I. T. Furuki et al. *Biochemistry* **50**, 7093-7103 (2011).

3G0924 微細パターン化モデル生体膜における膜結合タンパク質の濃縮の空間的制御

Spatially controlled accumulation of membrane-bound protein in a micro-patterned model biological membrane

Fumiko Okada¹, Kenichi Morigaki^{1,2} (¹Grad. Sch. Agr., Univ. Kobe, ²Res. Cent. Env. Genomi., Univ. Kobe)

Lipid rafts play a key role to regulate many cellular functions by recruiting membrane proteins. In a model system, liquid-ordered (*lo*) phases have been used as a model of lipid raft. We previously developed a micro-patterned model membrane on a solid support that can control the size and spatial distribution of *lo* phases (1). The model membranes comprised polymerized and fluid lipid bilayers. They induced phase separation of *lo* and liquid-ordered (*ld*) domains in a designed geometry because *ld* domains preferentially localized at the polymeric bilayer boundaries. The controlled phase separation should provide a means to purposefully concentrate membrane-bound proteins in defined regions. In the present work, we evaluated the accumulation of membrane-bound proteins in spatially defined areas. We used cholera toxin subunit B (CTB) as a model protein. The degree of CTB accumulation was correlated with the progress of phase separation, the ratio of polymeric/ fluid lipid bilayers, the geometries of polymerized bilayer regions, and the composition of fluid lipid bilayer. Spatially controlled accumulation of proteins should provide a novel approach to study lipid raft by separating membrane-bound proteins according to their affinities to lipid rafts. This technique should also pave a way toward sensitive bioassay and biosensor owing to the fact that protein molecules are accumulated in pre-defined regions.

(1) Okazaki et al. *Langmuir* 2010, 26, 4126

3G0936 圧力摂動熱量法によるジミリストイルホスファチジルコリン二分子膜の緩和挙動の解明への試み

An attempt to reveal the relaxation behavior of dimyristoylphosphatidylcholine bilayer by pressure perturbation calorimetry

Nobutake Tamai, Sayuri Kakibe, Saeko Tanaka, Masaki Goto, Hitoshi Matsuki (Dept. Life System, Inst. Technol. & Sci., Univ. of Tokushima)

Pressure perturbation calorimetry (PPC) is a relatively new calorimetric technique, which allows us to directly quantify the thermal expansion coefficient for solute molecules and colloidal particles in solution. In a PPC scan, the flow of the heat that occurs when a small isothermal pressure change (~ 5 bar) is applied to a sample solution is recorded as a function of time, and the expansion coefficient can be obtained from the total heat quantity transferred into the sample solution during the relaxation process by applying several thermodynamic relations. Therefore, the temperature dependence of the thermal coefficient is easily obtained by performing a series of PPC scans at different temperatures. Usually, this technique is used to study thermotropic volume behavior of solutes and dispersed particles and to estimate the relative volume changes of them with thermotropic physical transformations, such first-order phase transitions and conformational changes, on the basis of the temperature dependence of the thermal expansion coefficient. On the other hand, the time dependence of the heat flow measured in a PPC scan can provide us with the information about the relaxation dynamics of solutes and dispersed particles. On the basis of this idea, we have recently started the investigation on the relaxation process of a phospholipid bilayer membrane using the PPC technique. In this presentation, we will report on our recent results about the relaxation behavior of dimyristoylphosphatidylcholine bilayer membrane.

3G0948 リン脂質二重膜の圧力誘起指組み構造形成：疎水鎖長依存性と形成限界

Pressure-induced interdigitation of phospholipid bilayer membranes: dependence of acyl-chain length and limitation of the formation

Hitoshi Matsuki, Masaki Goto, Nobutake Tamai (Institute of Technology and Science, The University of Tokushima)

Bilayer membranes of diacylphosphatidylcholines (CnPC) are known to induce the interdigitated gel phase under high pressure. Since the interdigitated gel phase is extended into the low-pressure region with increasing the acyl-chain length, it is expected that the bilayers of CnPC with longer acyl chains induce the interdigitated gel phase under atmospheric pressure. In this study, we

investigated the bilayer interdigitation of CnPC with long acyl chains (Cn = 19-22). The temperature-pressure phase diagrams of four CnPC bilayers were constructed by differential scanning calorimetry and high-pressure light transmittance technique. We observed the interdigitation of the C19PC, C20PC and C21PC bilayers under high pressure, and the interdigitation was induced at lower pressures with the chain elongation. On the other hand, the C22PC bilayer showed the peculiar behavior: there exist two transitions under atmospheric pressure while one transition under high pressure. In order to confirm what the low-temperature phase of the C22PC bilayer is like, we performed measurements of the small angle neutron scattering and fluorescence spectroscopy under atmospheric pressure. These results proved that the phase is the interdigitated one. Therefore, it turned out that the maximum acyl-chain length required for the pressure-induced interdigitation is 21. Present results definitely indicate that the bilayer interdigitation is governed by the hydrophobic interaction as well as the hydrophilic interaction of the PC molecule in the bilayer.

3G1010 均でないリン脂質単分子膜における崩壊現象について

Collapse of nonuniform phospholipid monolayers

Masahiro Hibino, Ken Hashimoto, Takuya Fujisawa (Div. Appl. Sci., Muroran Inst. Technol.)

The behavior in the stochastic collapse of lipid monolayers of a mixture of dipalmitoylphosphatidylcholine and palmitoyloleoylphosphatidylglycerol, a model system of pulmonary surfactant assembly, has been studied extensively by using experimental techniques and theoretical models. The monolayers at the air-water interface appear to be phase separated under compression and retain the continuous liquid-expanded phase network surrounding islands of condensed phase even at a surface pressure approaching 70 mN/m. When the two-dimensional monolayers are compressed beyond the equilibrium surface pressure, they collapse and assume a three-dimensional formation. However, these phenomena have been observed under a condition of uniform monolayers. In fact, monolayer is not always uniform at the interface. We can rarely observe that the areas with the coexistence of LE-C phases are contiguous to each other, but distinct from each other by using fluorescence microscopy. The nonuniform surface density in the monolayer has an influence on the collapse. Here we investigate the collapse of compressed nonuniform binary phospholipid monolayers using Langmuir-isotherms, fluorescence microscopy and atomic force microscopy. The monolayers at the air-water interface underwent fast collapse by folding within 100 ms or slow collapse by the formation of Baumkuchen-like structure at high surface pressure. They were composed of bilayers that remained suspended beneath the film.

3G1022 脂質分子の電荷が引き起こす膜構造変化：2次元相分離と3次元曲率

Charge-induced transition in membrane mesoscopic structures: lateral domains and vesicular shapes

Hiroki Himeno, Tsutomu Hamada, Masahiro Takagi (School of Materials Science, Japan Advanced Institute of Science and Technology)

Living cells exhibit various 2D and 3D structural dynamics of lipid membranes. These structural changes are recognized as "membrane dynamics". The 2D membrane structures are represented by phase separation named "lipid raft". The 3D membrane structures are morphological changes, such as endocytosis and intercellular vesicular traffic. The intrinsic properties of constituent lipids should play an important role on such membrane dynamics.

Giant unilamellar vesicles (GUVs) are often used as a model of biological membranes, because they are large enough to be observed directly by optical microscope. Previously, using GUVs, we succeeded in reproducing 2D and 3D membrane dynamics such as phase separation (Hamada et al., *Soft Matter*, 7, 220-224, 2011) and vesicular formation (Hamada et al., *J. Am. Chem. Soc.*, 132, 10528-10532, 2010) by changing the molecular structure of membrane constituents.

To advance our understanding, we here focused on the effect of electric charges of lipid molecules, which is one of intrinsic properties of membrane lipids, on 2D (lateral phase separation) and 3D (membrane curvature) membrane mesoscopic structures.

3G1034 油中水滴エマルション遠心沈降法を用いたミクロスフェア内包型ジャイアントベシクル

Microsphere-containing giant vesicles prepared by water-in-oil emulsion centrifugation method

Yuno Natsume¹, Taro Toyota^{1,2} (¹*Grad. Art. Sci., Univ. Tokyo*, ²*JST PRESTO*)

In cells, biological macromolecules are contained at a volume fraction of 20-30 vol %. Recently, this situation has much drawn attention as a crucial factor of the crowding effect of the cytosol. To construct cell models, giant vesicles (GVs) confining macromolecules, microspheres, or both have been adopted. However, in the conventional methods such as the natural swelling method, the inner state (concentration, number density and so on) of macromolecules or microspheres is scarcely controlled and non-uniform among the GV. To form microsphere-containing GV as desired, we employed the water-in-oil (W/O) emulsion centrifugation method reported by Pautot et al. (Langmuir 2003, 19, 2870), because this method is based on W/O emulsion droplet as a template of GV. By using polystyrene microspheres (diameter = 1 μm) dispersions of 2.0–25 vol % to form W/O emulsions in liquid paraffin, we obtained GV that encapsulate microspheres with volume fractions in the range of 0–50 vol % and their diameters were in range of 4–40 μm . Although the distribution of the volume fraction of GV was broad, it should be noted that the highest volume fraction of GV was higher than that of W/O emulsion droplets. It is the advantage that we can achieve GV confining microspheres at high number density rather than that of microspheres dispersion we prepared initially. This technique can be applied to the GV encapsulation of other microspheres or macromolecules that exhibit entropy-driven effects, such as the crowding effect.

with a low pH buffer with a time-resolution of 100 ms. We observed that at the initial step the peak intensity of the L_α phase gradually decreased but at the same time that of the H_{II} phase gradually increased, indicating that the L_α phase directly converts into the H_{II} phase without formation of any intermediates. The rate constant of the initial step greatly depended on final pH and DOPS concentrations in DOPS/MO membranes.

[1] Adv. Planar Lipid Bilayers Liposome, 9, 163, 2009; Langmuir, 24, 3400, 2008, [2] J. Chem. Phys. 134, 145102, 2011

3G1110 X線及び電子線回折法を用いた皮膚角層の構造解析

Breakthrough for Unresolved Structural Problems in Skin Function by Combined Use of X-ray and Electron Diffraction Methods

Hiromitsu Nakazawa¹, Ichiro Hatta², Satoru Kato¹ (¹*Sch. Sci. Tech. Kwansei Gakuin Univ.*, ²*Nagoya Industrial Science Research Inst.*)

The outermost layer of the human skin, stratum corneum (SC), consists of terminally differentiated keratinocytes and highly ordered intercellular lipid layers. The SC serves as a physicochemical barrier between the inner body and the outer external environment. Notwithstanding the extensive studies on structure of intercellular lipids which plays a crucial role for the skin barrier properties, owing to lack of the structural evidence under functioning lot of unresolved problem are left open. Using X-ray and Electron diffraction methods we deepened understanding the healthy skin state and developing the percutaneous drugs.

Both synchrotron X-ray diffraction (XD) and electron diffraction (ED) methods are the very powerful tool to analyze the intercellular lipid organization in SC. The XD has higher resolution than the ED, but requires invasive operation because a large amount of skin sample is necessary to obtain reliable data. On the other hand, the ED makes it possible to analyze the structures of the SC noninvasively, but radiation damage by electron beam must be suppressed.

By best use of these methods we will report here a fundamental structural change of SC in the skin permeation of chemical agents, the effect of temperature in the skin permeation, and the regional structural differences on the body. We will present these results and future perspective for the SC structural study.

3H0900 GTP結合状態とGDP結合状態の微小管分子構造における大きな構造変化

Large Conformational Changes in Tubulin in the GTP- and GDP-States Microtubules Observed by Cryo Electron Microscopy

Hiroaki Yajima¹, Toshihiko Ogura², Ryo Nitta¹, Yasushi Okada¹, Chikara Sato², Nobutaka Hirokawa¹ (¹*Grad. Sch. Med., Univ. Tokyo*, ²*Biomedical Research Inst., AIST*)

Microtubules are dynamic polymers that stochastically switch between growing and shrinking phases and their dynamics is regulated by GTP hydrolysis by β -tubulin, but its mechanism remains elusive because high-resolution microtubule structures have only been revealed for the GDP-bound state. Here we solved the cryo-EM structure of microtubule stabilized with a GTP analogue guanylyl 5'- α , β -methylene diphosphonate (GMPCPP) at 8.8 Å resolution by developing a novel cryo-EM image reconstruction algorithm. In contrast to the crystal structures of GTP-bound tubulin relatives such as γ -tubulin and bacterial tubulins, significant changes were detected between GTP- and GDP-bound states at the contacts between tubulins both along the protofilament and between neighboring protofilaments, contributing to the stability of the microtubule lattice. These findings are consistent with the structural plasticity or lattice model, and suggest the structural basis not only for the regulatory mechanism of microtubule dynamics, but also for the recognition of the nucleotide state of microtubule by several microtubule-binding proteins, such as EB1 or kinesin.

3H0912 紡錘体は一方の極から他方へ構造変化を伝搬することで対称形状を維持する

Meiotic spindles maintain the symmetrical shape by propagating structural changes to the opposite side

Kazuya Suzuki¹, Jun Takagi¹, Takeshi Itabashi¹, Shin'ichi Ishiwata^{1,2} (¹*Dept. Phys., Adv. Sci. Eng., Waseda Univ.*, ²*WABIOS*)

A microtubule-based spindle is designed for proper chromosome segregation. Recent studies have demonstrated that bipolar spindle formation requires the force balance sustained by molecular motors. However, it remains unclear

3G1046 オイルフリー GUV に封入された分子演算システム RTRACS

Molecular computing system RTRACS encapsulated in oil-free giant unilamellar vesicle

Koh-ichiroh Shohda, Tadashi Sugawara, Akira Suyama (*Grad. Sch. Arts Sci., Univ. Tokyo*)

RTRACS (Reverse-transcription-and-Transcription-based Autonomous Computing System) is a molecular computing system in which RNA molecules work as input and output.^{1,2} RTRACS is a reaction network system which consists of modularized molecular logic gates containing the Boolean gate such as AND, OR, etc. We intend to construct a giant unilamellar vesicle (GUV) encapsulating RTRACS as a minimal model of living cell. At present, the best preparation method of GUV is the w/o emulsion centrifuge method.³ However the GUV prepared with the centrifuge method potentially contains the oil which surrounded the water droplet in the w/o emulsion. The oil changes the membrane properties, such as thickness, permeability, dynamics, etc. Hence we developed an oil-free GUV preparation method based on the lipid film gentle hydration method in the presence of mono- and di-valent metal cations. The inner water pool of GUV is stained with TMRA-dextran (M.W. 10,000) as a volume marker. The FAM-labeled molecular beacon probe which hybridizes with the output RNA molecule of RTRACS was used as a reaction marker. The dual-labeled GUV in which RTRACS runs was analyzed by optical microscopy and flow cytometry.

[1] N. Nitta, and A. Suyama *Lect. Note. Comput. Sc.*, **2004**, 2943, 203-212.

[2] M. Takinoue, D. Kiga, K. Shohda, and A. Suyama *Phys. Rev. E*, **2008**, 78, 041921.

[3] S. Pautot, B. J. Frisken, and D. A. Weitz *Langmuir*, **2003**, 19, 2870-2879.

3G1058 低い pH が誘起する DOPS/MO 膜の液晶相からキュービック相への相転移の初期過程

Initial Step of Low pH-Induced Lamellar to Bicontinuous Cubic Phase Transition in Dioleoylphosphatidylserine/Monoolein

Toshihiko Oka¹, Tomoki Takahashi¹, Taka-aki Tsuboi¹, Masahito Yamazaki² (¹*Fac. Sci., Shizuoka Univ.*, ²*Grad. Sch. Sci. Tech., Shizuoka Univ.*)

The modulation of electrostatic interactions due to surface charges of lipid membranes induces transitions between the L_α phase and the inverse bicontinuous cubic (Q_{II}) phase [1]. Using time-resolved small angle X-ray scattering (TR-SAXS) and a home-made rapid mixing method, we investigated the kinetics of low pH-induced L_α to Q_{II}^D phase transition in MLVs of dioleoylphosphatidylserine (DOPS)/monoolein (MO) in the presence of PEG-6K, and found that the H_{II} phase appeared after the pH change (the initial step), and then the H_{II} phase slowly converted into the Q_{II}^D phase (the second step) [2]. However, we could not follow the initial step due to the limited time-resolution of the method.

In this report, we investigated the initial step of the low pH-induced L_α to Q_{II}^D phase transition in DOPS/MO in the absence of PEG-6K using TR-SAXS with a stopped flow apparatus. We succeeded in following structural changes in the membranes after 100 ms of the mixing of DOPS/MO-MLV in a neutral buffer