we investigated the relation between protrusion/retraction activity and cell peripheral shape. Non-polarized keratocytes did not exhibit net translocation, however, active protrusion and retraction were observed around the cell periphery. The map of the cell peripheral activity showed that protrusion and retraction waves were traveling in opposite directions laterally along the cell boundary. Protrusion/retraction rate and protrusion/retraction persistence seemed to be correlated with the cell peripheral curvature. Biophysical bases of protrusion and retraction mechanism will be discussed on the basis of these results.

3P-170 シミュレーションと再構成系における ERK 二重リン酸化の双安定性

Numerical analysis and reconstitution of the dual-phosphorylation of ERK

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ERK, the mitogen-activated protein kinase (MAPK) in the Raf-MEK-ERK cascade, has two phosphorylation sites which are phosphorylated by MEK (MAPKK) and dephosphorylated by MKP. Bistability and hysteresis between the dual-phosphorylation state and the dephosphorylation state of ERK has been predicted in calculation assuming infinite volume of cells (Markevich et al., *JCB*: 353, 2004). In a numerical analysis using the Runge-Kutta method, we evaluated the bistability of ERK double phosphorylation. In the presence of 1000 nM ERK and 150 nM MKP, bistability of the ERK was observed at the MEK concentration in the range of 260-300 nM. But when ERK and MKP concentrations were reduced to be half, bistability was not observed at any concentration of MEK. This result suggests that ERK concentration is important for the bistability of ERK double phosphorylation. We reconstituted the ERK phosphorylation and dephosphorylation in *E. coli* cells, in which concentrations of ERK, MEK and MKP were independently controlled using extracellular chemicals. Protein concentrations were estimated by fluorescence intensities of Cerulean (a cyan fluorescent protein variant), Venus (a yellow fluorescent protein variant), and mCherry (a red fluorescent protein) each fused on ERK, MEK and MKP, respectively. The phospho-ERK concentration was assayed by antibody staining at individual cells. We are planning to compare the results of the simulation and experiments for the analysis of bistability predicted in the dual-phosphorylation of ERK.

3P-171 バイオサーファクタントの不飽和脂肪酸組成が正電荷リポソームによる遺伝子導入に及ぼす影響

Composition of unsaturated fatty acids in biosurfactants affects efficiency of gene transfection mediated by cationic liposomes

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Gene transfection is a fundamental technology for molecular and cell biology, and clinical gene therapy. Although, in the point of safety, several kinds of non-viral vectors for gene transfection have been investigated, further studies are still required to develop a non-viral vector which has comparable transfection efficiency to viral vectors. Recently, we found that a biosurfactant, mannosylerythritol lipid-A (MEL-A), dramatically increased the gene transfection efficiency by cationic liposomes via membrane fusion between the target cells and the cationic liposome. Here, we try to show the effect of lipid composition of MEL-A on gene transfection efficiency using three kinds of MEL-A-containing cationic liposomes which had unsaturated fatty acids (9.1%, 21.5%, and 46.3%) in MEL-A. We examined physicochemical properties of three kinds of MEL-A-containing cationic liposomes and observed the localization of these liposomes and DNA in the target cells using by laser scanning fluorescence microscopy. The efficiency of cationic liposomes with the MEL-A which had 21.5% unsaturated fatty acid, was highest in MEL-A-containing cationic liposomes. This was due to high membrane fusion activity and suitable form of liposome-DNA complexes for gene transfection in MEL-A (21.5%)-containing cationic liposomes. The results might be a help to develop not only MEL-A-containing liposomes but also other liposomes and some type of non-viral vectors to achieve high gene transfection efficiency in gene therapy.

3P-172 固体 NMR による抗菌ペプチド LFampinB の細菌模倣膜との相互作用と運動性に関する研究

Dynamics and interaction of bovine lactoferrampin with mimetic bacterial membrane as studied by solid-state NMR

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Lactoferrampin (LFampinB) is a new antimicrobial peptide identified in bovine lactoferrin (268-274 bLF). Since antimicrobial peptides work on the specific bacteria, structural information of antimicrobial activity is essential to understand antimicrobial activity and defensive mechanism of such peptides. In order to

understand the interaction between LFampinB and lipid, and to reveal dynamics of the peptide bound to lipids, we performed solid-state NMR measurements. Lipid components (DMPG: DMPC: CL=65:25:10 w/w) of mimetic bacterial membrane (mixed membrane) was used as a mimic of *staphylococcus aureus* membrane. The results of QCM measurements indicate that LFampinB showed greater affinity to negatively charged phospholipids (DMPG, CL) than neutral ones (DMPC). The result of K⁺ ISE measurements showed that LFampinB caused leakage of potassium ions across the acidic membranes. It is found that CL is critical for ion permeation. Solid-state ¹³C NMR was recorded and the results showed that spectral pattern was axially symmetric for [1-¹³C] Leu⁴, [1-¹³C] Ala⁷-LFampinB, indicating that LFampinB undergoes uniaxial rotation about the bilayer normal. It is summarized that LFampinB specifically interacts with acidic lipids to cause membrane defects to lead potassium ion permeation which is related to the antimicrobial activity. We are now examining ¹³C solid-state NMR measurements for other labeled sites to reveal detailed lipid-bound structure of LFampinB.

3P-173 固体 NMR と QCM による Mristoylated Alanine-Rich C Kinase Substrate と Phosphoinositides との相互作用解析

Interaction of Myristoylated Alanine-Rich C Kinase Substrate with Phosphoinositides in Phospholipid Membranes as studied by QCM and solid-state NMR

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Myristoylated alanine-rich C kinase substrate (MARCKS), a major protein-kinase C substrate, contains effector domain (151-175 : ED) in the middle of the molecule that interacts with various targets such as PI(4,5)P₂ and calmodulin. It plays a critical role as a crosstalk point on several signal transduction pathways. Phosphoinositides also play an important role in intracellular signaling cascade as a second-messenger. Cholesterols regulate membrane fluidity and promote "lipid rafts" mediating signal transduction pathways. In this work, association constants (K_a) between MARCKS-ED and PC bilayers containing PS and/or PI, PI(4,5)P₂ were measured by a QCM. The local structures of MARCKS-ED in lipid bilayers were examined using high resolution solid-state NMR. The result of QCM experiments clearly showed that MARCKS-ED had a high selectivity to multivalent-negatively charged lipid molecules (PI(4,5)P₂). ³¹P NMR signal showed the well resolved axially symmetric pattern, indicating that MARCKS-ED stabilizes the membrane surface by neutralizing negative charge of acidic lipids with Lys residues. In the ¹³C NMR spectra, the signals of [1-¹³C]Phe and [2-¹³C]Gly shown the two chemical shift values, indicating that β-strand and random coil conformations co-exist as free and bound states. Cholesterols regulated the magnetic field orientation of lipid membranes, and consequently it was noticed that line widths were broadened as a result of decreasing dynamics of lipid membranes. In addition, cholesterol caused conformational change in the presence of PI(4,5)P₂. The hydrophobic residues of MARCKS-ED may insert into the bilayers as a result of strong electrostatic interaction between acidic lipids such as PI(4,5)P₂ and the basic residues in the N- and C- terminal region of the peptide.

3P-174 ¹³C NMR によるオピオイドペプチドダイノルフィンとκ-受容体細胞外第2ループの相互作用解析

Interaction of opioid peptide dynorphin with extracellular loop 2 of κ-opioid receptor as studied by ¹³C NMR

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Opioid peptide dynorphin is known to specifically interact with κ-receptor, and it shows the morphine like activity. Therefore, the structural analysis of this system can lead to a design of new pain relief drugs. However, large scale expression system of the receptor has not established yet. We, therefore, tried to reveal the interaction of κ-receptor with dynorphin using fragment of the extracellular loop 2 (ECL-2:196-228) of κ-receptor. Previous solid-state NMR study revealed that dynorphin interact with ELC-2 at the location of Val¹⁰-Ala¹⁵. However, ¹³C NMR signal of [1-¹³C]Val¹⁰ was not separated well from that of lipid in the large bicelle system. Therefore, in this study, we used the small bicelle to obtain well resolved ¹³C NMR signals. [1-¹³C]Val¹⁰, [3-¹³C]Ala¹⁵-ECL-2 and dynorphin was synthesized by means of solid phase method. These peptides were incorporated into the small bicelle system (DMPC:DHPC molar ratio of 1:1) with lipid:peptide:dynorphin molar ratio of 200:1:(x=0,1,2,5). ¹³C-NMR spectra of these membrane samples were recorded on Bruker DRX400 NMR spectrometer. The spectral resolution of ¹³C NMR for the small bicelle system was improved dramatically as compared with that of the large bicelle system. Consequently, the ¹³C NMR signals of [1-¹³C]Val¹⁰-ECL-2 were separated well from those of lipids. Since the chemical shift value of [1-¹³C]Val¹⁰ was shifted 2.7ppm by the addition of dynorphin, it turned out that [1-¹³C]Val¹⁰ is interacted with dynorphin. These result showed that Val¹⁰ to Ala¹⁵ of ECL-2 interact with the κ-helix part of Dynorphin.