2P272 抗菌性ペプチドマガイニン2が脂質膜に形成するポアの特性解析
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Characterization of the pore in lipid membranes induced by antimicrobial peptide, magainin 2
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Using the single GUV method, we found that low concentrations (3-10 μM) of magainin 2 induced rapid leakage of a fluorescent probe, calcein, from the inside of single 50% DOPG/50% DOPC-GUVs, indicating that magainin 2 formed pores in the membrane, and then succeeded in revealing elementary processes of the pore formation. In this report, to characterize the magainin 2-induced pore in lipid membranes, we investigated the effect of lipid compositions on the pore formation and size of the pore.

An increase in cholesterol concentration in the DOPG/DOPC-membrane with the same surface charge density greatly decreased the probability of the magainin 2-induced pore formation. However, the presence of ceramide in the membrane did not greatly decrease that of the pore formation. On the basis of the results, we discuss the effect of cholesterol. Next, to investigate the pore size, we investigated interaction of magainin 2 with single GUVs containing various fluorescent probe-labeled dextrans and proteins. 15 μM magainin 2 induced a rapid leakage of 4 kDa dextran from single GUVs, but it induced a very slow and incomplete leakage of 10 kDa dextran. In the case of FITC-BSA, it induced a transient, but very small (~10%) leakage. It may be due to the contamination of unlabeled FITC in the FITC-BSA. Preliminary results suggest that the pore size increased with magainin 2 concentration. In the conference, we will show the detailed data using various sizes of dextran and proteins, and discuss the pore size.

[1] *Biochemistry*, 44, 15823, 2005

2P274 抗菌性ペプチドマガイニン2のポア形成に脂質膜の表面電荷密度がおよぼす効果
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2aK06 Effects of Surface Charge Density of Lipid Membranes on the Pore Formation Induced by Antimicrobial Peptide, Magainin 2
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Interactions of antimicrobial peptides with lipid membranes have been investigated using a liposome suspension, and their details remain unclear. Using the single GUV method, we have succeeded in revealing elementary processes of the pore formation in lipid membranes induced by magainin 2 [1]. In this report, to elucidate the mechanism of the magainin 2-induced pore formation, we investigated the effect of surface charge density of membranes on the pore formation.

To change the surface charge density, we controlled negatively charged DOPG concentration in DOPG/DOPC membrane from 30 to 60 mol%. We found that, in all kinds of GUVs, magainin 2 induced a rapid leakage of calcein from single GUVs, showing that magainin 2 formed pores in the membrane. For GUVs with the same charge density, the fraction of leaked GUV, P , increased with time, and P at a fixed time increased with magainin 2 concentration. The magainin 2 concentration at $P = 0.5$ at a fixed time increased with a decrease in the surface charge density. We obtained binding constants of magainin 2 to various DOPG/DOPC membranes under the same condition. By considering that magainin 2 distributed in a buffer according to the Gouy-Chapman theory, we obtained magainin 2 concentration in the membrane interface, X_s . On the basis of these results, we discuss the role of magainin 2 concentration in the membrane interface in the magainin 2-induced pore formation.

[1] *Biochemistry*, 44, 15823, 2005

2P273 エピガロカテキンガレートが誘起する PC-GUV の破裂に対するコレステロールの効果
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Effect of Cholesterol on the (-)-Epigallocatechin Gallate-Induced Burst of PC-GUVs
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Tea catechins such as (-)-epigallocatechin gallate (EGCg) have been considered to have antibacterial activity and antioxidant. Several investigations indicated that lipid membranes are one of the targets of catechins for their activities. However, the detail interaction of catechins with lipid membranes remained unclear. In our previous report [1], using the single GUV method, we found that low concentrations of EGCg (60 μM) induced a rapid leakage of a fluorescent probe, calcein, from the inside of a single egg PC-GUV, which changed into a small lump of lipids after the leakage. The strong correlation between the leakage and the burst of GUVs indicated that the leakage of calcein occurred as a result of the burst of the GUV. On the basis of results of several experiments, we have proposed the mechanism of the EGCg-induced burst of vesicles.

In this report, we investigated the effect of cholesterol (chol) on the EGCg-induced bursting of GUVs. At first, we investigated effect of EGCg on DOPC-GUVs. Low concentrations of EGCg (30 μM) induced bursting of GUVs, which is similar to that of egg PC-GUVs. On the other hand, EGCg could not induce the burst of DOPC/chol (6/4: molar ratio)-GUV at a concentration > 100 μM . For egg-PC/chol (5/5)-GUVs; no burst occurred at a concentration 1mM EGCg. In the conference, we will show a detailed dependence of the burst of PC-GUVs on their cholesterol concentration, and discuss the mechanism of the inhibition of the burst of GUVs by cholesterol.

[1] *Biophys. J.* 92, 3178-3194, 2007.

2P275 脂質膜の水透過性とそれに対するアクチン細胞骨格の効果
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Water permeability of lipid membranes of GUVs and its dependence on actin cytoskeletons inside the GUVs
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Response of cells to osmotic pressure and control of water permeability of biomembranes are very important for cells to survive. There have been many studies using cells, but their details remain unknown. It is reported that actin filaments decreased the osmotic pressure-induced water flow through a semipermeable membrane [1]. However, it is well not known whether actin filaments and actin/filamin A-gel in cells can change water permeability of biomembranes or not. In this study, we investigated water permeability of various lipid membranes of GUVs by the micropipet aspiration technique, and then its dependence on actin cytoskeletons inside the GUVs.

At first, we measured water permeability of DOPC membrane in the L_α phase. When we transferred a single DOPC-GUV fixed by a micropipet using a small aspiration pressure into a buffer with a higher osmolarity, the volume of the GUV decreased due to the water efflux. We determined the decrease in the GUV volume by the increase in aspiration length in the micropipet. We measured the time course of volume change of the GUV, and analyzed it quantitatively to get water permeability of DOPC-membrane, P_{app} . It was $45 \pm 4 \mu\text{m/s}$. Then we measured water permeability of DPPC/cholesterol (6/4, molar ratio) membrane in a liquid-ordered phase; P_{app} was $1.3 \pm 0.1 \mu\text{m/s}$, which was much smaller than that of DOPC membrane. In the conference, we will show data of the effect of actin cytoskeletons on water permeability of lipid membranes, and discuss it.

[1] *J. Phys. Chem.* 110, 13572-13581, 2006