2P248 ホスファチジルイノシトールのラフト局在化に対するステロールの影響
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Effect of sterols on the localization of phosphatidylinositol into the sphingomyelin and sterol mixed domain
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Recently a simple view of a membrane as a fluid mosaic has been reassessed with the possible presence of rigid raft domains which are composed of specific lipids such as sphingolipids, glycolipids, and sterols. Glycophosphatidylinositol(GPI)-anchored proteins are also localized into the rafts and involves in cellular signal transduction. However, incorporation mechanism of GPI-anchored proteins into raft domains remains to be known. We have reported that cholesterol (Chol) molecule may promote the localization of phosphatidylinositol (PI) into raft domains. Here we investigated the interaction between PI and plant sterols such as stigmasterol (Stig) on the basis of surface pressure (π)-area (A) isotherm measurements.

Addition of PI into Stig monolayers shifted π -A isotherms toward higher mean molecular areas. The deviations of experimentally obtained mean molecular areas from theoretically calculated areas on the basis of ideal mixing were negative under physiological pressure ($\pi=30$ mN/m). These results indicate that intermolecular accommodation is induced between PI and Stig molecules. The condensing effect of Stig on PI monolayer was similar to that of Chol, suggesting that Stig molecules play a critical role in the localization of PI into the raft domain in plant plasma membranes.

2P250 ウシラクトフェリシンと酸性リン脂質二重膜との相互作用およびその抗菌作用に及ぼすカルジオリピンの影響
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The Effect of Cardiolipin in Selective Interaction of LfcinB with Acidic Phospholipid Bilayers and its Antimicrobial Activity
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Bovine lactoferricin (LfcinB) is an antimicrobial peptide which consists of 25-amino acid residues with the sequence FKRRWQWRMKKLGAPSTCVRRAF. The previous study revealed that LfcinB interacted selectively with acidic phospholipid bilayers (APB) with the weight percentage of 65% DMPG, 10% cardiolipin (CL) and 25% DMPC as a mimic of cell membrane of *Staphylococcus aureus* and molecular mechanism of the antimicrobial activity could be attributed to the pore formation in the bacterial membrane induced by LfcinB. In this study, we used non-CL APB with the weight percentage of 72% DMPG and 28% DMPC. The interaction of LfcinB with this non-CL APB was investigated by means of QCM measurements and solid-state NMR spectroscopy. Moreover, we inquired into the effect of CL to the molecular mechanism of the antimicrobial activity of LfcinB by means of potassium ion selective electrode by examining the pore forming properties. QCM measurements showed that the association constant of LfcinB with CL-included APB was similar to that with non-CL APB. Although CL was two negatively charged, the affinity of LfcinB for APB was not enhanced by CL. ³¹P DD-static NMR spectra indicated that large isotropic peaks at 0 ppm appeared in LfcinB-CL-included APB systems. Defects in lipid bilayers may be formed to cause isotropic components in the APB at 40°C. However, they did not appear in LfcinB-non-CL APB systems. It turned out that CL played a role to make defects in lipid bilayers.

2P249 電子線回折による生体皮膚角層の非侵襲構造解析
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Structural analysis of the skin stratum corneum by electron diffraction
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The main role of the skin stratum corneum (SC) is to serve as a physicochemical barrier between the body and the environment. The barrier function is chiefly performed by highly ordered intercellular lipid lamellae in the SC. Recent X-ray and electron diffraction studies have suggested that the lateral packing structures of the intercellular lipids play a crucial role for the skin barrier function. We have developed a noninvasive electron diffraction method to observe the lateral packing structures of the intercellular lipids easily at room temperature. Introduction of a beam shutter and a supersensitive imaging plate as a detector reduced the electron beam damage and made it possible to obtain clear diffraction images without a cryoholder. We applied this method to analyzing the lipid organizations in the SCs collected from human cheek, forearm and forehead. A significant difference was observed in the frequency of appearance of hexagonal and orthorhombic hydrocarbon chain packing between the SC lipids from these three regions; the quantitative ratio of orthorhombic to hexagonal domains in the forearm SCs was higher than those in the forehead and cheek SCs. We also measured the transepidermal water loss (TEWL) at each region and examined the correlation between the lipid packing structure and TEWL. We will discuss the role of the lateral organization of SC lipids in the skin barrier function.

2P251 固体NMRによる β -endorphinとコレステロール含有不飽和脂質膜の相互作用解析
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Interaction of β -endorphin with a model membrane consisting of unsaturated lipids and cholesterol bilayers as studied by solid-state NMR
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β -endorphin is an endogenous opioid peptide which mainly exists in mammalian central nerve system and has an analgetic effect by binding to opioid receptors. The purpose of this study is to reveal a bioactive function of β -endorphin in the molecular level. Peptide to lipids molar ratio was adjusted to 1:20. The molar ratio of POPC:POPE:POPS was prepared to 35:35:30. Additionally 9%, 20% and 30% cholesterol was added to the mixed lipid samples. ¹³C and ³¹P solid state NMR spectra were recorded on a Chemagnetic CMX400 Infinity NMR spectrometer. It was revealed that β -endorphin bound to mixed phospholipids bilayers formed α -helix around Gly³ and α -helix and mixed structures of random coil around Leu¹⁴ and random coil around Ala²⁶. It was further revealed that Gly³ site of β -endorphin kept forming α -helix in a variety of molar ratios of cholesterol. By contrast, interaction between β -endorphin and phospholipids increased by adding cholesterol. Signal of Gly³ and Ala²⁶ sites appeared in ¹³C DD-Static NMR spectra and the intensities were stronger than those of Leu¹⁴ site in ¹³C DD-MAS NMR spectra. This result indicates that binding of central domain of β -endorphin to membrane is stronger and less mobile than those of C- and N-terminal domains. ¹³C NMR spectra of lipid components were measured for the mixed bilayer samples containing peptide. ¹³C NMR lines of membrane surface region in lipid molecules decreased by adding β -endorphin. This result indicates that β -endorphin preferentially interacts with membrane surface region.