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Mechanism of liposome leakage induced by fatty acid divalent cations

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It has been reported that a permeability transition in liposomes was induced in the presence of Ca^{2+} and palmitic acid and this might play a key role in the process of apoptosis. We have reported the occurrence of phase separation in supported bilayers of phosphatidylcholine containing anionic lipids and suggested that this might increase the permeability. In this study we applied scanning probe microscopy (SPM) and fluorometry to investigate the physicochemical properties as well as structures and interactions of the liposomes and the effects of the presence of fatty acids and divalent cations. For the preparation of liposomes, conventional method of freezing/thawing at -10/30 ℃ of the lipid suspension followed by filtration was used. Substances to be loaded inside the liposomes were contained in the buffer solutions. The resulting unilamellar vesicles were further purified by gel filtration chromatography. In SPM observation, topological, error, and frictional images were obtained both in atmospheric environments and in aqueous solutions. Fusion induced by the addition of divalent cations was observed between fluorescently-labeled liposomes by the fluorescence energy transfer. This was further supported by the observation of fluorescence enhancement following the mixing of encapsulated solutes (DPA and Tb^{3+}). Corresponding SPM images were also observed. Emergence of an anomalous bump on the liposome surface was observed when calcium ions were added. The effects of other fatty acids and divalent cations are discussed.

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Structure and Dynamics of Raft Model Membrane

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A common feature of lipid microdomains, so-called rafts, is their peculiar lipid composition, being rich in glycosphingolipids (GSLs), sphingomyelin and cholesterol. Gangliosides, major components of GSLs, are composed of a ceramide linked to an oligosaccharide chain with sialic acids. The functions of rafts relate closely to the peculiar features of GSLs. By using neutron and X-ray scattering techniques we have clarified functional characteristics of GSLs and GSLs-containing lipid mixtures. 1) GSLs form ellipsoidal micelles. Temperature change hydrates or dehydrates sugar head region with accompanying the bending and the change of charge of sugar chains. 2) GSLs predominantly locate at outer-leaflet of the ganglioside-phospholipid vesicle. 4) In GSL-cholesterol systems there exists the maximum miscibility of cholesterols to gangliosides, and the cholesterol-dependent micelle-to-vesicle and Ca-induced vesicle-to-lamellar transitions occur due to interdigitated structure formation between the sugar chain. 5) Hydration and bending of sugar heads dominate dynamics of GSL micelles. The bending modulus of GSL-cholesterol-phospholipid vesicles takes a smallest value at the lipid composition as similar as in intact membrane including rafts. 6) Water permeability is controlled by potassium ion through GSL-domains. Thus, the GSL microdomains can modulate locally charge and hydrophilicity of membrane surfaces and dominate dynamics of membranes, which are essential for accumulation and activation of functional proteins in plasma membrane.

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Spontaneous Formation of a Solid-Supported Mixed Bilayer Composed of Phospholipid and Nonionic Surfactant under an Aqueous Medium

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We investigated about spontaneous formation of mixed bilayer composed of a phospholipid and a nonionic surfactant on a hydrophilic solid substrate under an aqueous medium containing the phospholipid and surfactant. We particularly focused on the correlation between the composition in the solid-supported mixed bilayer and in the mixed micelle. On a basis of this study, the properties of a model biological membrane could be regulated reversibly by the composition of an aqueous solution.

Octylglucoside (OG) and dioleoylphosphatidylcholine (DOPC) were used as the surfactant and phospholipid, respectively. Dynamic light scattering (DLS) was used to study the mixed micelle formation in the solution. An OG/DOPC adsorption layer was deposited on a germanium or mica substrate by only dipping the substrate in an aqueous OG and DOPC solution. The properties of the adsorption layer were studied by ATR-FTIR spectroscopy and atomic force microscopy (AFM).

The DLS measurements showed that, as the size of the mixed micelle decreased, the fraction of OG in the mixed micelle and the concentration of monomeric OG increased. This indicates that the chemical potential of OG increases with the fraction of OG in the mixed micelle, as well as that of DOPC decreases. On the other hand, formation of a mixed bilayer on a substrate in equilibrium with the mixed micelle solution was confirmed by ATR-FTIR spectroscopy and AFM. DOPC molecules were distributed into the mixed bilayer in preference to OG, compared with the case in the mixed micelle.

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Supported phospholipid bilayers on SiO_2 and TiO_2 surfaces: Effects of surface chemical species and atomic structures

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Lipid bilayer membranes deposited on solid surfaces are called "supported planar bilayers" (SPBs), and expected to be an effective cell-membrane-mimicking model system in vitro. We investigated the influence of surface properties on the SPB formation and characteristic.We prepared SiO2 surfaces with different surface hydroxyl groups (-OHs) density by annealing the SiO2 layer on Si(100). Irreversible thermal desorption of -OHs reduced the surface hydrophilicity. The formation rate of dimyristoylphosphatidylcholine (DMPC)-SPB from 100-nm-filtered vesicles was faster on less hydrophilic surfaces. We proposed that a stable hydrogen-bonded water layer on the SiO2 surface worked as a barrier to prevent vesicle adhesion on the surface. The water layer stability and its dependence are discussed from theoretical calculation results using Si-O cluster models. The surface -OH density little affected the fluidity of once formed SPBs, which was measured by the FRAP method. Application to the area-selective SPB deposition using surface patterning by the focused ion beam will also be described.We also investigated the effects of surface atomic structures and photo-induced hydrophilicity on single-stepped rutile-TiO2 low index surfaces. Single-stepped TiO2(100) surface was prepared by HF aq. treatment and annealing at 700°C at O2 flow. The DMPC 50-nm-filtered vesicles transformed to planar bilayer on the O2-annealed TiO2(100), but adsorbed as vesicles on the UV-irradiated TiO2(100). Trace of single steps was recognized on the SPB on the TiO2(100).

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