Title	静電作用による脂質二分子膜小胞の秩序形成メカニズムの解明：2次元相分離構造と3次元膜孔形成のカップリング
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Abstract

The basic structure of biomembrane is lipid bilayer composed of various types of lipid molecules. Biomembranes exhibit various two-dimensional (2D) and three dimensional (3D) dynamics, and play a very important role in regulation of cellular function. The 2D dynamic is represented by phase separation called "lipid raft formation". The 3D membrane dynamics are morphological changes such as endo- exocytosis, vesicular transport, and autophagy. In these membrane dynamics, physicochemical properties of lipid membranes should play a very important role as well as protein function. In order to reveal the physicochemical properties of lipid membranes, giant unilamellar vesicles (GUV) consisting of mixtures of lipids and cholesterol have been used as model biomembranes.

In the past, most of the studies have investigated the primary physical property of lipid membrane in uncharged model systems. However, biomembranes also include negatively charged lipids. As physicochemical properties of lipid membranes, charged lipids are one of the important factors to be considered. In addition, surface charge of membrane could be controlled by cellular ion such as sodium ion, and calcium ion.

In this thesis, we clarify the electric charge effects on the 2D dynamics (phase behaviour) and 3D dynamics (membrane morphology). We also explored the salt screening effect on charged membranes. We discussed the effects of charge on membrane 2D and 3D dynamics based on free energy model. First, we investigated the phase separation induced by negatively charged lipids. As compared to the neutral lipid mixtures, the phase separation is suppressed by charged unsaturated lipid, whereas it is enhanced by charged saturated lipid. The phase behaviors of all charged mixtures approach that of the neutral mixture due to screening of electrostatic interactions by adding the salt.

Second, we investigated the localization of cholesterol and phase behavior in various mixtures of charged lipid membranes. We found that cholesterol prefers to be localized in unsaturated charged lipid rich phase, while does not to be localized in saturated charged lipid rich phase. In presence of salt, localization of cholesterol was changed significantly. These results suggest that the interaction between charged lipid and cholesterol plays an important role in structural regulation of phase separation in lipid membrane.

Third, we investigated about the effect of charge on membrane 3D dynamics and discussed relationship between membrane 2D and 3D dynamics. Although vesicles basically formed spherical shape in neutral lipid mixtures, pore formation structures were observed in charged lipid mixtures. Fraction of pore formation vesicles increased with charged lipid concentration. In presence of salt, pore formation was suppressed. In addition, we reproduced experimental system using Coarse-grained molecular dynamics (MD) simulations. MD simulation showed that charged lipids stabilized the edge of membrane pore. Hence, 2D phase separation between neutral lipid and charged lipid is very important to from 3D pore formation.

Our findings will be advanced understanding of the mechanism of dynamic process in biomembrane such as 2D dynamics of lipid rafts structure, and 3D dynamics of membrane morphology including endocytosis, autophagy, and vesicular transport during the signal transduction. Moreover, our finding may help to understand the mechanisms that play an essential role in the interactions of proteins with lipid mixtures.

Key words: phospholipid, liposome, electric charge, phase separation, morphological change