Abstract

[Background]

Lipid rafts on biomembranes are thought to have essential functions in signal transduction in living organisms. The structure and function of lipid rafts are expected to contribute to the understanding of cellular functions. Phospholipids, the main components of biomembranes, spontaneously form lipid bilayer structures in water. The liposome, which is closed to the lipid bilayer, has attracted much attention as a model system because its structure is similar to that of biomembranes. Further, the phase separation formed in liposomes consisting of multicomponent phospholipids is helpful as a model system for raft formation studies. From this point of view, studies on phase separation at the liposome membrane surface have been conducted, and many studies have been researched to control the phase separation behavior by using electrically neutral phospholipid membranes.

However, considering the biological environment, the presence of charged lipids, the induction of phase separation under isothermal conditions, and the roles of metal ions and polyamines inside and outside the cell are all critical. Previous studies have reported phase separation of phospholipid membranes containing charged lipids by the addition of metal ions and phase separation induced by osmotic application of the hypotonic solution to neutral lipid membrane vesicles. In conjunction with these studies, we use a system that more closely resembles the biological environment, considering the effects of the presence of charged lipids, isothermal environments, variable temperature environments, the addition of metal ions and polyamines, and the valence of charged lipids.

[Objective]

In this thesis, we clarify the changes of phase separation on the DOPS/DPPC binary GUVs in a hypotonic solution under isothermal conditions. We investigated the formation of the phase separation of DOPS/DPPC/Chol trinary GUVs adding monovalent to pentavalent metal salts and amines at room temperature, 30℃, 40℃. Also, we discussed the effect of multivalent charged lipids on the membrane behavior based on line tension calculations.

[Results]

First, phase separation was induced by osmotic pressure on the GUVs, and a three-phase coexistence structure was observed: a DPPC-rich phase, a negatively charged DOPS [DOPS(-)] phase, and a neutral DOPS [DOPS(N)] phase. The ionic dissociation of the DOPS head group was found to be essential for phase separation. Next, as the concentrations of metal ions and amines increased, phase separation formation was promoted. It was found that phase separation was more likely to occur at room temperature than at 30℃, 40℃. On the other hand, the concentration at which phase separation was induced significantly varied depending on the type of metal ions or amines. The concentration required for phase separation was temperature-sensitive for amines, which are linear chains. Last, confirmed that charged lipids decreased the domain line tension and that the addition of $CaCl_2$ suppressed the decrease in line tension. It was clarified that electrostatic interaction is involved in the decrease of line tension.

These experimental results suggest that the degree of ionization of charged lipids, osmotic pressure, temperature change, structure and concentration of added salts (especially metal ions and amines), and lipid valence significantly affect raft formation. Our findings may be helpful for the contribution of understanding the formation of ordered structures in living organisms and can be a model for charged lipid membrane.

Keyword: lipid; charged lipid; phase separation; osmotic pressure; salts; polyamines; line tension