Title	脂質膜における相分離構造の線張力による制御
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Abstract

[Background]

Lipid bilayer membrane is the basic structure of cell membrane which is the boundary between inside and outside regions of a cell. Cell membranes have lateral heterogeneity consisting of various lipids, proteins, glycolipids, and glycoproteins. From the raft hypothesis, raft regions mainly consisting of sphingolipids and cholesterol are believed to regulate membrane-protein interaction, cell signaling, and membrane trafficking by modulating the lateral organization of the membrane. Phospholipids in water naturally assemble into the bilayer structure by hydrophobic interactions. Interestingly, phase separation can occur in the artificial multicomponent lipid bilayers. The typical phases at room temperature are a solid-ordered (S_o) phase which is rich in saturated lipids, a liquid-ordered (L_o) phase which is rich in saturated lipids and cholesterol, and liquid-disordered (L_d) phase composed largely of unsaturated lipids.

It is important to consider the interfacial tension at the phase-separated domain boundary (line tension) when we discuss the stability of phase separation. Generally, as the line tension becomes smaller, the domain size becomes smaller, the domains start to fluctuate, and the phase separation disappears finally. Therefore, the control of line tension leads to the control of phase-separated structures.

In this thesis, the line tension is controlled by two methods. First one is osmotic swelling. The response of cells and their phase behaviors to the osmotic stress is important for cell homeostasis and survival in several body systems, particularly in the circulation system. It was reported that osmotic swelling can enhance the phase separation to occur. In other words, the osmotic swelling may increase the line tension. However, the physicochemical mechanism behind still unclear. Second method is the addition of unsaturated fatty acids. Unsaturated fatty acids decrease the high-density lipoprotein cholesterol and may decrease the risk of cardiovascular disease. Also, Oleic acid, one of unsaturated fatty acids, decreases the line tension at liquid domain boundary. It is expected that the more precise control of phase separation becomes possible by combining these two methods.

[Objective]

In this thesis, we investigated the effects of osmotic pressure on phase separation and miscibility temperature in lipid membranes consisting of dioleolylphosphocholine (DOPC)/ dipalmitoylphosphocholine (DPPC)/ Cholesterol (Chol). The line tension analysis at the domain boundary of DOPC/DPPC/Chol and diphytanoylphosphatidylcholine (DiphyPC)/DPPC/Chol were also compared. In the last part, we showed the modulate of the line tension in the fatty acids mixed DOPC/DPPC/Chol membrane. Also, the changes of line tension in fatty acids-containing lipid membranes by osmotic pressure were examined.

[Results]

It was found that the application of osmotic pressure can widen the area of phase-separated in the phase diagram and increase the miscibility temperature which is a transition temperature between phase separation and homogeneous phase. Interestingly, we found that miscibility temperature increased greater at the higher cholesterol concentration. Furthermore, osmotic pressure can increase the line tension at the domain boundary in both DOPC/DPPC/Chol and DiphyPC/DPPC/Chol systems similarly. The plausible mechanism behind was also proposed that the osmotic pressure mediated membrane tension suppresses the membrane fluctuation and causes the change of free energy.

In the last part, we showed the effects of chemical stimuli, in terms of different chain length and cis position of five monounsaturated fatty acids. Based on their physical properties and our finding results, we can categorize their behaviors in lipid membrane into two types: Oleic acid type which the fatty acid can include in the DPPC region and disturbed the ordered chain length leading to lower in line tension, and Palmitic acid type which can exclude cholesterol from the L_o phase and produce the S_o phase. We also showed their effects under osmotic pressure which can increase line tension in the mixed lipid membranes.

Our findings may provide insight into biophysics of cell membranes how cell response to osmotic pressure without osmotic shock and how unsaturated fatty acids regulate the line tension and can be an insightful model for the understanding of the human cell membrane.

Keywords: osmotic pressure, phase separation, line tension, miscibility temperature, unsaturated fatty acid