Abstract

【Background]

Cells sense the extracellular environment and initiate physiological responses to maintain homeostasis. Membrane receptors convert extracellular information into intracellular information by interacting with signal molecules. Dysregulation of signal transduction causes various diseases. Obesity and aging, which are abnormal states of lipid quality and quantity, are major risk factors for various diseases. Thus, it is possible that changes in lipidome affect the interaction between lipids and biological membrane properties and disturb signal transduction, eventually causing various diseases. However, the effect of lipid interactions and membrane properties on signal transduction is poorly understood. Lipid interactions and properties affect membrane dynamics. There are many kinds of membrane dynamics. We have classified these membrane dynamics into 2 types; 3D membrane dynamics (endocytosis, autophagy, exocytosis) and 2D membrane dynamics (lipid rafts). However, the biological membrane is not suitable for the analysis of lipid interaction because the components of the biological membrane are very complicated. Thus, in this thesis, we used biomimetic membranes (liposomes) because they can be prepared with simple compositions and are large enough to be observed by microscopy. We have studied 2D and 3D liposome dynamics. We have shown that liposomes cause deformation by adding surfactants and classified the 3D membrane dynamics depending on the strength of irritation. The evaluation method enables us to evaluate the irritancy of surfactants even when the irritancy of the surfactants is low. Liposomes can be changed their lipid compositions and phase-separated structures can be formed; liquid-ordered phase (Lo) rich in saturated lipids and cholesterol, liquid-disordered (Ld) phase rich in unsaturated lipids, and solid-ordered (So) phase rich in saturated lipids. This phase separation can be found in living cells. The So phase occurs in cholesterol (Chol)-poor endoplasmic reticulum (ER) and the Lo phase occurs in cholesterol-rich membranes such as cell membranes.

[Objective]

The purpose of this study was to visualize physiological responses using biomimetic membranes.

In chapter 2, we investigated the irritation of sodium cocoyl glutamate by an irritating evaluation system with 3D membrane dynamics. Furthermore, we examined the correlation between the irritancy evaluation system and the stinging test. In particular, the relationship between the carbon chain length of sodium glutamate and irritancy was examined. In chapter 3, we regarded So/Ld and Lo/Ld liposomes as ER membrane-model and raft model, respectively, and investigated the effect of two forms of vitamin E, α-Tocopherol(Toc) and α-Tocotrienol(Toc3), on amyloid β adsorption on these model membranes.

[Results]

In chapter 2, by observing membrane dynamics after adding amino acid surfactant to liposome solution, we found that comparing the irritancy of sodium cocoyl glutamate with that of its main component, sodium lauroyl glutamate, it was found in both liposome and stinging test that sodium lauroyl glutamate was the more irritating. The irritancy of sodium lauroyl glutamate was reduced by amphoteric surfactants. Furthermore, we established an evaluation system for Flip-Flop rate, which is important for membrane deformation. In chapter 3, we found that Toc and Toc3 do not affect the Lo/ Ld phase separation. DSC measurements suggested that affinity between Chol and VE (Toc and Toc3) is low, and VE is mainly incorporated into Ld phase in raft model membrane. Furthermore, DPH anisotropy measurement shows that VE increased the order of Ld phase. As the result, line tension of Lo/Ld phase boundary was decreased by adding VEs. Toc and Toc3 decreased the phase separation of So/Ld phase separation. DSC measurement using DPPC/VE binary system suggested that VE decreased the interaction between DPPCs. The effect was more strongly for Toc3 than Toc. Thus, the amount of Toc which is incorporated into So phase was larger than Toc3. We found that inhibition of So phase by Toc and Toc3 decreases amyloid β adsorption onto ER membrane model. Our experimental results show that changes in lipids interactions are closely related to physiological responses, and that visualization by 2D and 3D membrane dynamics can serve as a model system for various physiological responses. Furthermore, combined with superior image processing systems, we will be able to understand physiological responses in even greater detail.

[Keyword]

Liposome, Membrane deformation, Surfactant, Phase separation, Amyloid-β