別紙４－１（課程博士（英文））

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Abstract (Doctor)

Title of Thesis	Non-raft submicron domain formation in lipid bilayers induced by polyunsaturated lipids and its application to reconstitution of membrane proteins

Approx. 800 words

Cell membranes are complex matrices composed of a large variety of lipids and membrane proteins, serving as functional barriers between cells and their environment. Apart from providing some structural support for cells, they constitute reaction fields for the transportation of materials, signals and energy into and out of cells through the membranes. Therefore, cell membranes are responsible for several cellular processes, such as immunity, metabolism and infection. Various types of lipids exist in cell membranes. Their organization affects the dynamics and physical properties of the lipid bilayer membranes. These membrane characteristics in turn are responsible for the functions of the cell membranes. The formation of two-dimensional lipid domains due to preferential association of specific lipid molecules and membrane proteins has been widely studied to understand the mechanism of cell membrane reaction and related pathologies. The representative lipid domain is the "raft", which has been extensively investigated using artificial lipid bilayer systems.

In this Ph.D. thesis, I aimed to clarify the formation mechanism of a newly discovered non-raft domain in lipid bilayers, aiming to apply the domain as a platform for the reconstitution of ion channels into artificial lipid bilayers. The non-raft domain formation in this study is a new concept, postulating a possibility of the existence of such domains in cell membranes. The formation mechanism of this non-raft domain is worth elucidating from the viewpoints of physical chemistry of lipid self-assembly and engineering of biomembranes. I also aimed to improve the membrane fusion efficiency of proteoliposome (PL) into artificial lipid bilayer which is vital for ion channel studies. In my Ph.D. study, the ion channel that I focused on was human *ether-a-go-go* related gene (hERG) channel, a voltage-dependent potassium channel. Many drugs with chemically diverse structures exhibit adverse effect on the hERG channel, resulting in potential cardiac arrhythmias. Hence, observing its active structure at physiological condition is desirable for more accurate drug design to avoid the adverse effect, and thus is valuable from the viewpoint of medical and pharmaceutical for membrane protein studies.

First, I clarified the composition and mechanism of the non-raft domain formation in lipid bilayer comprising two types of lipids with low phase transition temperature (Low-T_m) and cholesterol (Chol), by

fluorescence microscopy and atomic force microscopy (AFM). Low-T_m lipid is in the liquid crystalline phase at the ambient temperature of experiments (~25 °C). Two different Low-T_m lipids homogeneously mixed at any ratio. Chol-induced domain formation in these completely miscible lipids is a unique phenomenon to be discovered. I prepared ternary supported lipid bilayers (SLBs) using egg-derived phosphatidylcholine (eggPC), Chol, and one of three different types of phosphatidylethanolamine (PE): egg-derived PE, 1-palmitoyl-2-oleoyl-PE (16:0-18:1PE), and 1,2-didocosahexaenoyl-PE (22:6-22:6PE). The non-raft domains were observed as depressions in the AFM topographies. Their area fraction (θ) increased with concentration of PE, and 22:6-22:6PE yield the largest θ among the three PEs, suggesting the non-raft domains in the PE+eggPC+Chol-SLBs were enriched with polyunsaturated PE.

Next, I thoroughly elucidated the formation mechanism of the non-raft domain by clarifying the role of polyunsaturated acyl chains. I explored the effects of the degree of unsaturation and the double bond distribution at the PE *sn*-position on the formation of the non-raft domains. The ternary SLBs containing PE with various degrees of unsaturation were investigated by the fluorescence microscopy, AFM, and force–distance curve measurement. The area fraction of the non-raft domain increased with the concentration and degree of unsaturation of PE, suggesting the importance of the double bonds at the acyl chains for the non-raft domain formation. The force-distance curve measurement shows that the non-raft domains were in the liquid-disordered-like state, whereas their surrounding regions were in the liquid-ordered-like state. The results suggest that the segregation of PE from Chol-rich region, which mainly comprises monounsaturated PC and Chol, induces a non-raft domain formation. I also found that polyunsaturated PC is also capable to induce the non-raft domain formation, depending on its degree of unsaturation and concentration. I proposed the mechanism for the formation of these non-raft domains based on molecular interactions involving the hydrophobic and hydrophilic parts of the bilayer membrane.

Then, I investigated the effects of 22:6-22:6PE, which induced the largest θ, on the fusion efficiency of PLs into PE+PC+Chol-SLB. The findings clearly show that the large area of non-raft domains in SLB induced by 22:6-22:6PE drastically improved the PL fusion efficiency, compared to the effects of eggPE on the PL fusion efficiency. This enables a promising method for the reconstitution of membrane protein into artificial lipid bilayer.

Lastly, I observed hERG channel molecules by AFM at physiological condition. Pure hERG channel with full-length amino acid residues was synthesized using cell-free translation system (by courtesy of Prof. Yuzuru Tozawa). I reconstituted the hERG channels in SLB and observed their association states at physiological condition. The molecular images of hERG channels in various oligomeric states were successfully observed, and their association states were examined quantitatively. This study is expected to be valuable and useful for drug design to avoid drug-induced side effects on hERG channel.

In summary, this Ph.D. thesis describes the composition and mechanism of non-raft domain formation, its application to improve PL fusion efficiency into artificial lipid bilayer, and the molecular images of hERG channel at physiological condition. These research works are expected to stimulate further studies on lipid domains in biological membrane, and to contribute to the fields of interface chemistry, and pharmaceutical and medical industries.