別紙４（課程博士）

Date of Submission:
平成　29 年　1 月　10 日

Department 環境・生命工学専攻	Student ID Number 学籍番号	第 093506 号
Applicant's name 氏名	岡本　吉晃	

Supervisors 指導教員	手老　龍吾 岩佐　精二 伊津野　真一

Abstract

論文内容の要旨　(博士)

Title of Thesis 博士学位論文名	Development of new artificial biomembrane systems and their characterization based on mesoscopic morphology and lateral diffusion （新規人工生体膜系の開発およびメソスケール領域における構造と側方拡散性に基づいた特性評価）

(Approx. 800 words)

(要旨　1,200 字程度)

Lipid bilayer is a fundamental structure of plasma membranes, and behaves as the reaction field for various membrane reactions through the organization of lipid domains and the molecule diffusion of biomolecules. The behavior of lipids and membrane proteins existing in lipid bilayers are important targets to elucidate the elementary processes of membrane reactions. Therefore new experimental systems and methods for the membrane reactions are demanded. Supported lipid bilayer (SLB), which is one of artificial lipid bilayer systems, has been used as a cell membrane model to study the physicochemical properties of lipid bilayer, and as a platform to investigate the structure and the function of membrane proteins. Recently, graphene oxide (GO), which is one of the graphene derivative with oxygen functional groups such as hydroxyl, epoxy, and carboxyl groups on its surface, is exploited for the biological application as a fluorescence quencher with unique properties. Also synthetic fluorinated lipids and surfactants which are substituted their hydrogen atoms in the hydrophobic alkyl chain with fluorine atoms are applied in the field of biochemistry and medicine for extraction and reorganization of the membrane proteins according to biological and chemical inertness of fluorocarbons. I expected that application of these materials, GO and fluorinated lipids to the SLB system leads to the development of new methods and experimental systems for the measurement of the behavior of biomolecules such as lipids and membrane proteins in the lipid bilayer. Herein, I studied the development of the new artificial lipid bilayer systems using GO and a fluorinated lipid for the measurement of biomolecule behavior in lipid bilayer. I evaluate their structures and physicochemical properties based on the mesoscopic morphology and the lateral diffusion in SLB.

Graphene and GO have characteristic fluorescence quenching abilities compared with other general quencher molecules: independent of the wavelength of fluorescence probes; long effective range. GO exhibits the amphiphilicity, because GO has a heterogeneously surface consisting of oxidized hydrophilic regions and nanoscale hydrophobic regions with graphene structure. The fundamental study on the effects of GO with the microscopically heterogeneous surface on the physicochemical property of lipid bilayer is an important subject for the application of GO in the field of biology and nanobiotechnology. As a first step of my work, we formed the SLB of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) on GO by the vesicle fusion method. GO was prepared according to the modified Hummer's method, which is one of the common chemical exfoliation methods. I evaluated the structure and fluidity of the SLB formed on GO by the atomic force microscopy (AFM) and the fluorescence recovery after photobleaching (FRAP) measurement. I proposed the structural model of the SLB/GO system, and found that second layer of SLB which is not formed by the vesicle fusion method generally, was stably formed on GO.

To evaluate the fluidity of SLB formed on GO quantitatively, I conjugated quantum dots (Qdots) as a bright fluorescence probe to the SLB surface covalently for the single particle tracking (SPT) measurement. I also introduced a lipid conjugated with polyethylene glycol (PEG) chain on its head group in DOPC-SLB to suppress the non-specific adsorption of Qdot to SLB surface. I observed the lateral diffusion of the Qdot-conjugated lipids in the SLB on GO even under the effect of fluorescence quenching by GO. I found several Qdot-conjugated lipids diffused between the GO region and SiO_2 region, and evaluated the diffusion coefficient (D) of the Qdot conjugated-lipid in the GO and SiO_2 regions on the basis of the mean-square displacement (MSD) analysis. The average value of D of the Qdot-conjugated lipids on the GO region was approximately 30% smaller than that on the SiO_2 region. I investigated the morphology and fluidity of SLB containing PEG-lipid on SiO_2/Si substrates with and without GO. Depression domains were observed in the SLB and increased with the concentration of PEG-lipid on SiO_2/Si without GO, and the depression domains were localized on GO on the SiO_2/Si with GO. These results suggest that GO under SLB concentrated PEG-lipid, and as a result D was smaller in the GO region than in the SiO_2 region.

The fluorinated lipid affects to lipids and membrane proteins in a different manner from the common phospholipids with hydrocarbon chains. I prepared the SLB of a novel partially fluorinated lipid 1,2-di-(11,11,12,12,13,13,14,14,14-nonafluorotetradecanoyl)-sn-glycero-3-phosphocholine (F4-DMPC), and evaluated the lateral lipid mobility and its temperature dependence as the index to estimate the intermolecular interaction of the partially fluorinated lipid. Fluorination of lipid alkyl chain weakened the intermolecular interaction in the hydrophobic core in the lipid bilayer, and I evaluated its degree qualitatively on the basis of the activation energy of lateral diffusion obtained from Arrhenius plots of D.

In these works, I constructed new artificial lipid bilayer systems applying GO and fluorinated lipids, and revealed their structures and physicochemical properties. I believe that these systems become a foundation for the experimental method for the highly accurate measurement and for the control of the position, orientation, and activity of biomolecules in lipid bilayer membranes. I expect that the fundamental study of lipid bilayer using the new materials such as GO and fluorinated lipid lead to greater understanding for the membrane reaction. It is expected that the further development of these new artificial lipid bilayer systems to reveal the elementary steps of the membrane reactions through the recognition of the asymmetry of lipid bilayer, the detection of the microscopic change of the thickness of lipid bilayer, and the expression of a new function of membrane proteins.