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Concentration Effect of Graphene Oxide on Microdomains in Multicomponent Lipid Bilayer Membranes

Yoshiaki Okamoto[1*], Shun Saito[1], Seiji Iwasa[1], Ryugo Tero[1,2]

[1]Department of Environmental and Life Sciences,
[2]Electronics-Inpired Interdisciplinary Research Institute (EIIRIS),
Toyohashi University of Technology, Tenpaku-cho, Toyohashi, 441-8580, Japan.
Phone: +81-(0)532-81-6816 E-mail: y093506@edu.tut.ac.jp

Abstract

Lipid bilayer is the fundamental structure of plasma membranes, and behaves as the reaction field for various membrane reactions. Recently we established the formation of supported lipid bilayers (SLBs) on graphene oxide (GO) with the vesicle fusion method, and evaluated the fluidity of SLB on GO by the single particle tracking. In this study, we observed the multicomponent SLBs formed on GO, and we found that GO has the property to concentrate specific lipid domains on GO.

1. Introduction

A lipid bilayer is a fundamental structure of plasma membranes, and play important roles for membrane reactions such as the transport of material, information, and energy into and out of cells through the molecular diffusion and the organization of lipid domains. Supported lipid bilayers (SLBs), which are artificial bilayer membranes at solid-liquid interfaces, have been investigated as cell mem-brane models to study the physicochemical properties of lipid bilayers, and used as the platform for membrane proteins. One of advantages of SLB systems is that surface functions of the solid substrates are available to control the properties of lipid bilayers. Recently, various biological applications of graphene and graphene oxide (GO) were reported [1]. Interaction between GO and various kinds of biological molecules, such as DNA, RNA and proteins. Previously we prepared SLBs on GO by vesicle fusion method, and evaluated its fluidity by the single particle tracking (SPT) method using quantum dot (Qdot) as a fluorescence probe [2]. The SPT measurement of the SLB of dioleoylphosphatidylcholine (DOPC) containing 5% PEGylated lipid showed that the diffusion coefficient (*D*) of the SLB formed on GO was lower than that of SLB on SiO_2/Si [2]. In this study, we found that the lipid domains of PEGylated lipids were concentrated on GO. We investigated the lipid concentration effect of GO by the observation of SLB containing the pegylated lipid and of multicomponent SLB system with an atomic force microscope (AFM) and a fluorescence microscope.

2. Experimental

The GO suspension was prepared by the modified Hummer's method [3], and was dropcast on a thermally oxidized SiO_2/Si substrate. The chloroform solution of DOPC mixed with dipalmitoylphosphatidylthioethanol (DPPTE) at the molar ratio of $1:10^{-8}$, and PEGylated lipid (PEG-DSPE) was dried with N_2 flow, followed by overnight evacuation. Then we prepared the vesicle suspension of the mixed lipids by suspending the vacuum-dried lipid film into buffer solution. The vesicle suspension of DOPC mixed with dipalmitoylphosphatidylcholine (DPPC) and a fluorescence labeled lipid (Rb-DOPE) at the molar ratio of 1:1:0.2 was prepared by the same protocol. We prepared the SLB on the GO-deposited SiO_2/Si substrates by the vesicle fusion method [4], and observed with fluorescence microscope and AFM in a buffer solution (100 mM KCl, 25 mM HEPES, 5 mM $CaCl_2$, pH7.4/ NaOH).

3. Results and Discussion

In order to study the effect of the addition of PEG-DSPE for the SLB formed on GO, we observed the surface of SLB containing 5% PEG-DSPE using AFM. Figure 1 shows the AFM topography of the DOPC-SLB containing 5% PEG-DSPE formed on GO. Depression domains were observed on the GO region, while the SLB on the SiO_2 region was flat and uniform. In our previous work, the topology of the DOPC-SLB without PEG-DSPE formed on GO was planar [5]. Therefore, we suppose that the depression domains in the GO region were originated from PEG-DSPE.

We investigated the effect of the concentration of PEG-DSPE on the structure and fluidity of DOPC-SLB on a bare SiO_2/Si substrate without GO. Figure 2 shows AFM topographies of DOPC-SLB containing PEG-DSPE at the molar ratio of 2.5% - 7.5%. The SLB was flat and uniform

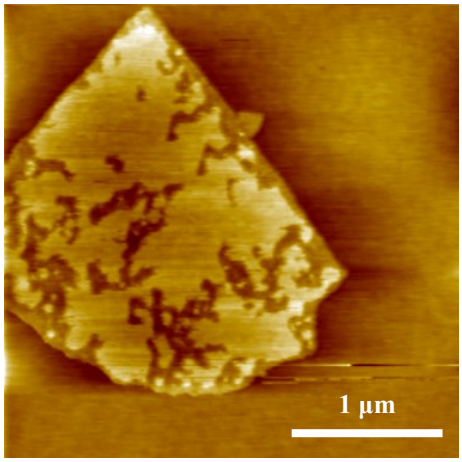
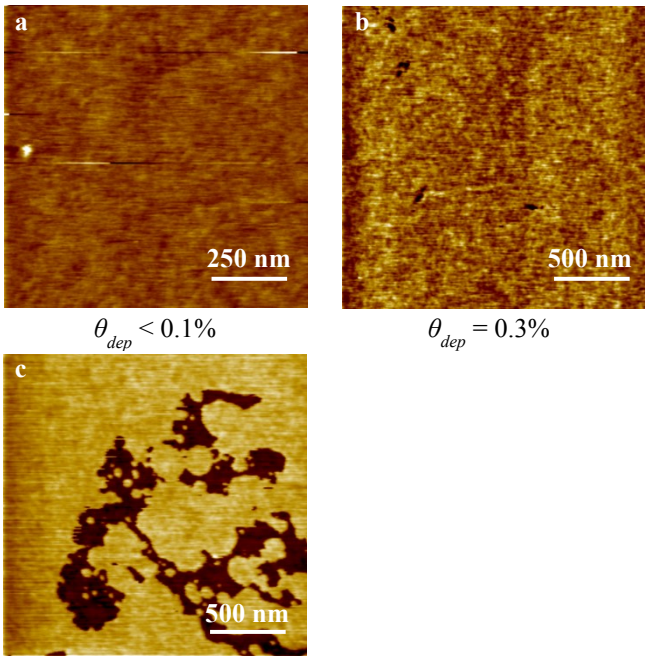


Fig. 1 AFM topography of DOPC-SLB containing 5% PEG-DSPE formed on the GO/SiO_2/Si substrate.



$\theta_{dep} < 0.1\%$ $\theta_{dep} = 0.3\%$

$\theta_{dep} = 17.9\%$

Fig. 2 AFM topographies of DOPC-SLB containing PEG-DSPE at the molar ratio of (a) 2.5%, (b) 5.0%, and (c) 7.5% formed on SiO_2/Si substrate.

at 2.5% (Fig. 2a), but depression domains were observed at 5.0% (Fig. 2b) and 7.5% (Fig. 2c). The area fraction of the depression domain (θ_{dep}) at each PEG-DSPE concentration was $\theta_{dep} < 0.001$ at 2.5%, $\theta_{dep} = 0.003$ at 5.0%, $\theta_{dep} = 0.179$ at 7.5%. Correlation between θ_{dep} and the concentration of PEG-DSPE indicates that the depression domain was derived from PEG-DSPE. Therefore we attribute the depression domains on GO shown in Fig. 1 to the PEG-DSPE-derived domain, which was concentrated on GO. In the SLB with 5% PEG-DSPE in Fig. 1, $\theta_{dep} = 0.298$ on GO. These results show that PEG-DSPE was concentrated on GO and as a result the microdomains derived from PEG-DSPE were localized on GO.

We measured D of SLB containing PEG-DSPE on the bare SiO_2/Si at each molar ratio corresponding to Fig. 2. The values of D were 2.64 $\mu m^2/s$ at PEG-DSPE 2.5%, 2.45 $\mu m^2/s$ at 5.0%, and 1.21 $\mu m^2/s$ at 7.5%. Lateral mobility of lipids in SLB with the concentration of PEG-DSPE, thus the increase in θ_{dep}. It is consistent with our recently report in which D in the DOPC-SLB with 5% PEG-DSPE is approximately 30% smaller on GO than on SiO_2 region. GO has an effect concentrating rigid PEG-DSPE-rich domains on it, and the SLB around GO was "purified" containing higher concentration of DOPC.

To investigate the concentrating effect of GO further, we formed the bicomponent SLB of DOPC and DPPC, in which phase separation between DPPC-rich gel (solid) phase and DOPC-rich liquid crystal (L_α) (fluid) phase. The former is observed higher in AFM topography and darker in fluorescence image than the latter. In the AFM topography of DOPC+DPPC-SLB (Fig. 3), we found that the gel-domains

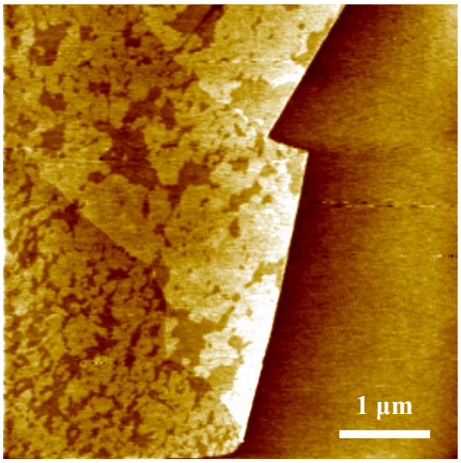


Fig. 3 AFM topography of DOPC+DPPC-SLB formed on the GO/SiO_2/Si substrate

existed on the GO region only and that the surrounding SiO_2 region was free from the gel-domains. Fluorescence images also showed that the dark gel-domains were not observed in the vicinity of GO while the gel-domains existed in the SiO_2 region away from GO. These results suggest that GO concentrates the domains of rigid gel-domains, as with the case of PEG-DSPE.

4. Conclusions

We formed the multicomponent SLBs of DOPC+PEG-DSPE and DOPC+DPPC on GO. The AFM topographies clearly showed that GO concentrated rigid domains, PEG-DSPE- or DPPC-rich domains, leaving fluid DOPC-rich SLB on the surrounding SiO_2 region.

Acknowledgements

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