

Fluidity Evaluation of Cell Membrane Model Formed on Graphene Oxide with Single Particle Tracking Using Qdot

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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 for the development of a new method to measure the behavior of biomolecules in lipid bilayers using GO. In this study, we conjugated quantum dots (Qdots) to the SLB surface, and evaluated the fluidity of the SLB on GO with single particle observation. We found several diffusing Qdots on the SLB on GO, and obtained the diffusion coefficient from their trajectories.

1. Introduction

A lipid bilayer is a fundamental structure of plasma membranes which play important roles for membrane reactions such as the transportation of material, information, and energy into and out of cells. Supported lipid bilayers (SLBs), which are artificial bilayer membranes at solid-liquid interfaces, have been investigated as cell membrane models to study the physicochemical properties of lipid bilayers, and used as the platform for membrane proteins. Recently, various biological applications of graphene and graphene oxide (GO) were reported [1]. For example, GO was applied to the sensing of biological binding such as DNA hybridization, antigen-antibody reaction, aptamer binding, and so on. We have established a fabrication protocol of SLBs on graphene oxide (GO) for the development of a new method to measure the behavior of biomolecules in the plasma membrane model using graphene [2]. We showed that the SLB formed on GO has fluidity from the result of fluorescence recovery after photobleaching (FRAP) measurement, while we could not evaluate its fluidity quantitatively because of the strong fluorescence quenching of GO.

In this study, we conjugated quantum dots (Qdots) as a brighter probe than dye-molecules to the surface of SLBs, in order to evaluate the fluidity of the SLB on GO.

2. Experimental

The GO suspension was prepared by the modified Hummer's method [3], and was dropcast on a thermally oxidized SiO₂/Si substrate. The chloroform solution of dioleoylphosphatidylcholine (DOPC) mixed with a fluorescence-labeled lipid (Rb-DOPE: Ex/Em=557/571 nm) or

dipalmitoylphosphatidylthioethanol (DPPTE) at the molar ratio of 1: 10⁻⁸ was dried with N₂ 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. We prepared the SLB on the GO-deposited SiO₂/Si substrates by the vesicle fusion method [2,4], and observed with epi-fluorescence microscope and atomic force microscope (AFM) in a buffer solution (100 mM KCl, 25 mM HEPES, 5 mM CaCl₂, pH7.4/NaOH). A carboxyl-coated Qdot was modified by a maleimide-hydrazide hetero-cross linker and 2-(2-aminoethoxy) ethanol (AEE) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and was added to SLB containing thiol-terminated lipid. Single molecule observation was performed with an inverted fluorescence microscope with a diagonal illumination setup, which achieves the single molecule imaging on an opaque silicon wafer [4].

3. Result and Discussion

Based on the AFM topography and the FRAP measurement of the DOPC-SLB containing Rb-DOPE on the SiO₂/Si substrate covered with GO, we proposed the structural model of SLB/GO/SiO₂ system, and found that not only single lipid bilayer but also double lipid bilayers were formed on GO [2]. Then we performed the fluorescence single molecule observation of the surface of the SLB formed on GO. However, we could not detect the fluorescence signal from the Rb-DOPE in the SLB on GO because of the high efficiency of the quenching by GO.

Therefore we used a Qdot as a brighter fluorescence probe than dye molecules. We conjugated the carboxyl-terminated Qdot to the SLB surface through the modification of the Qdot surface with the hetero-cross linker. We observed the fluorescence signal of the Qdots even on GO, but the Qdots did not diffuse probably because the modified Qdots non-specifically adsorbed to the surface of the SLB and/or because the modified Qdot bound with several thiol-terminated lipids in the SLB. We mixed AEE with the cross linker to control the number of the maleimide group on the Qdot surface. We observed the several diffusing Qdots on the SLB surface, even though many immobile Qdot still existed. We evaluated the diffusion behavior of the Qdot-conjugated lipids into the SLB formed on SiO₂/Si by the mean-square displacement (MSD) analysis. The average of diffusion coefficient (*D*) was ~0.5 μm²/s (Fig.1a), while the *D*-histogram showed wide distribution (Fig.1b).

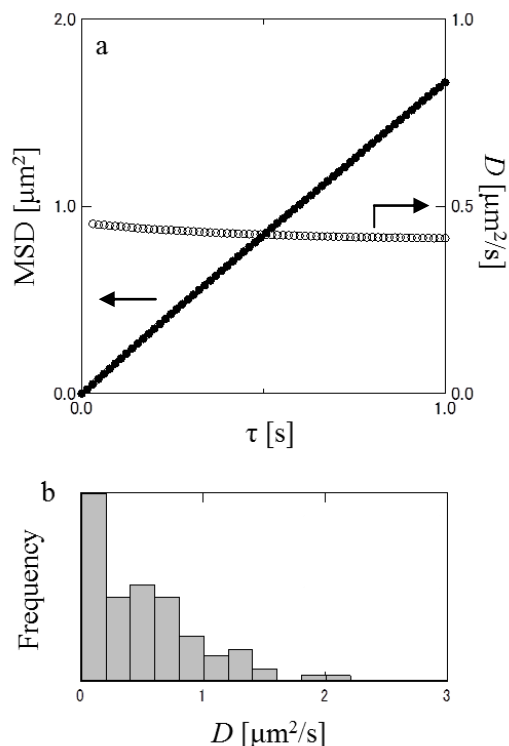


Fig.1 (a) MSD- τ plot (black circle) and D - τ plot (white circle) obtained from the average of 90 trajectories of Qdot-conjugated lipids in the SLB on SiO_2 . (b) Histogram of diffusion coefficient obtained from each trajectory.

This result suggests that multivalent bond were formed between the Qdots and the SLB. Because the diffusion coefficient was independent of the time interval (τ) in D - τ plot, we found that the Qdot-conjugated lipids diffused by the Brownian motion. We conjugated the Qdot to the SLB formed on GO. We also obtained a few diffusing Qdots on the SLB on GO, while more immobile Qdots existed on the GO region than the SiO_2 region (Fig.2a). We obtained a long trajectory enough to reliably evaluate the diffusion coefficient by the MSD analysis from one Qdot particle because of the long fluorescence life time of Qdots. The diffusion coefficient obtained from the single Qdot-conjugated lipid on the GO region was $\sim 0.6 \mu\text{m}^2/\text{s}$, and D - τ plot showed that the diffusion behavior was the Brownian motion as with the SLB formed on SiO_2/Si (Fig.2b). These results suggest that the SLBs formed on the GO have the similar physicochemical property of the SLBs formed on SiO_2 .

4. Conclusions

We formed the SLB on GO and evaluated its diffusion behavior by the single particle tracking using the Qdot as a fluorescence probe. We conjugated covalently Qdot modified with hetero-cross linker to the SLB surface, and measured the diffusion of Qdot-conjugated lipids by single particle tracking method. We found that the fluidity and its spatiotemporal dependence of the SLB formed on GO was similar to that on SiO_2/Si .

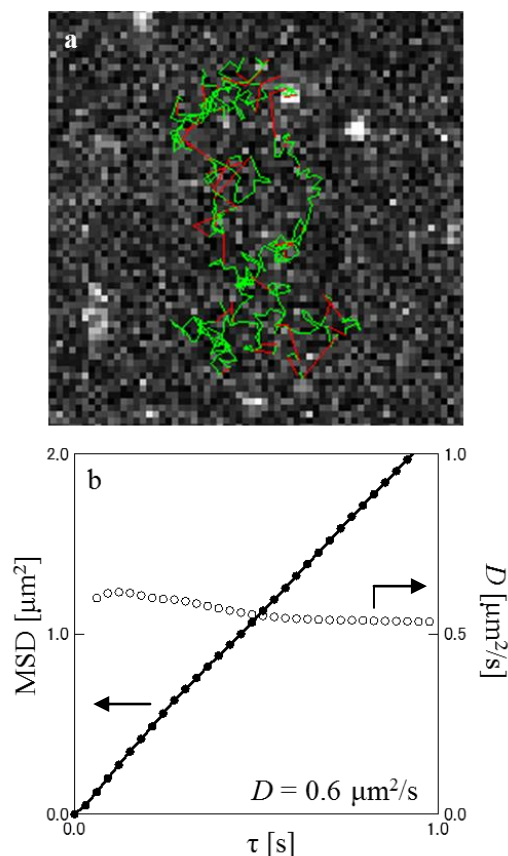


Fig.2 (a) Fluorescence single particle image ($10 \times 10 \mu\text{m}^2$) and the trajectory of Qdot-conjugated lipid in the SLB on GO. (b) MSD- τ (black circle) and D - τ (white circle) plot of the single Qdot in (a).

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