Extended Abstracts of the 2014 International Conference on Solid State Devices and Materials, Tsukuba, 2014, pp292-293

Formation and Fluidity Measurement of Artificial Lipid Membranes on Polyvinyl Chloride Substrate

Takuji Kobayashi[1*], Akiteru Kono[1], Toshiaki Hattori[1], Kazuaki Sawada[1], Ryugo Tero[2,3**]

[1]Department of Electrical and Electronic Information Engineering, [2]Department of Environmental and Life Sciences,
[3]Electronics-Inspired Interdisciplinary Research Institute, Hibarigaoka Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
Phone: +81-(0)532-81-5138, *E-mail: kobayashi-t@int.ee.tut.ac.jp, **tero@tut.ac.jp

Abstract

We prepared an artificial lipid membrane on a plasticized poly(vinyl chloride) (PVC) substrate. The planar lipid membrane of 1,2-dioleoyl-sn-glycero-3 -phosphocholine (DOPC) was formed on the PVC substrate by the vesicle fusion method. The observation with a conventional epi-fluorescence microscope and a confocal laser scanning microscope gave geometrically uniform images of the lipid membrane on the PVC membrane. The fluidity and the mobile fraction of the lipid membrane was evaluated by the fluorescence recovery after photobleaching method, and compared with that on a thermally oxidized SiO_2/Si substrate. lipid membrane on the PVC membrane contained immobile fraction ~30%, but the diffusion in the mobile fraction was two times faster than that in PLM on SiO_2/Si, which had little immobile fraction.

1. Introduction

Plasticized poly(vinyl chloride) (PVC) is the most commonly used polymer material for the membranes for ionselective electrodes. Incorporation of ionophores, cage-type molecules selectively capture specific kinds of ions, into the PVC membranes achieved highly selective detection of K^+, Na^+, Ca^{2+}, etc. Such ion-selective PVC electrodes have been widely used and provided valuable information. An interdisciplinary example of the application of the ion-selective PVC membrane is the ion sensors based on field effect transistor (FET) and charge-coupled device (CCD) [1].

Ion concentrations around cell membranes are key factors for the signal transduction into and out of cells across the cell membranes. Bimolecular sheets of amphiphilic phospholipid molecules, called lipid bilayers, are the fundamental structure of cell membranes. Proteins in cell membranes including ion channels retain their proper structures and activities only in the lipid bilayers. Platforms and experimental techniques for the researches of membrane proteins using artificial lipid bilayer membranes are demanded, because the membrane proteins occupy ~60% of the targets of drug discovery. Lipid bilayers are artificial lipid membranes existing at the interfaces between solids with hydrophilic surfaces and aqueous solutions. The lipid membranes on solid sensing devices will be valuable as artificial biomembrane platforms for the investigation of lipids and membrane proteins in vitro.

2. Experimental

Preparation of lipid membranes

In this study, we fabricated lipid membrane on a PVC substrate using the vesicle fusion method [2]. We used a Si wafer with a Si_3N_4 layer deposited with low-pressure chemical-vapor deposition (LP-CVD), mimicking the ion-sensing Si_3N_4 layer of the CCD ion sensor developed by K. Sawada and co-workers [1]. The PVC/THF solution was dropcast on a Si_3N_4/Si substrate, and annealed at 60°C for 3 h in an electric oven. The vesicle suspension of DOPC doped with dye-labeled lipids (2-(β-BODIPY 530/550)-1-hexadecanoyl-sn-glycero-3-phosphocholine (BODIPY-HPC) or 1,2-dipalmitoylsn- glycero-3- phosphoethanolamine-N- (lissamine rhodamine B sulfonyl) (Rb-DPPE)) was prepared as follows. The vacuum dried-film of the lipid mixture at the required amount and ratio was suspended in a buffer solution (100 mM KCl, 25 mM HEPES/NaOH (pH 7.4) at the lipid concentration of 0.4 mM. The vesicles in the suspension were transformed to unilamellar vesicle through the processes of frozen-and-thawed, extrusion through a 100-pore polycarbonate filter, and sonication.

The PVC and thermally oxidized SiO_2/Si substrates were immersed in the vesicle suspension and incubated at 45°C for 1 h. The excess vesicles in the liquid phase were washed out by exchanging the suspension by the fresh buffer solution before the fluorescence microscope observation and the FRAP measurement.

Fluorescence Microscopy and Fluorescence Recovery after Photobleaching

We observed the PVC and SiO_2/Si substrates after the lipid membrane formation with an epi-fluorescence microscope (Olympus BX51WI) to check the whether the vesicles transformed to lipid membrane or adsorbed as vesicles on the substrate surfaces. The sample was irradiated by a 200W Mercury vapor short arc lamp through a 530-550 band-pass filter. The three-dimensional image of the lipid membrane on PVC was obtained by using a confocal laser scanning microscope (CLSM) (Nikon, A1) with the 561 nm laser as the excitation light source. The FRAP measurement was performed with the CLSM. The photobleaching was performed within 2 ms with the 1778 time stronger laser power than that for the observation, and the size of the photobleached area (S) was 26.7×26.7 μm^2.

3. Results and Discussion

The two-dimensional diffusion coefficient (*D*) and the mobile fraction (A) were obtained. Figure 1(a) shows the

epi-fluorescence images of the PVC substrate after the incubation in the DOPC vesicle suspension. Lipid membranes, in the state of either lipid membrane or adhesive vesicles, exist at the gray region occupying the majority of the surface. Qualitative FRAP observation (Fig. 1b-1d) showed that the fluorescence signal in the gray region recovered with time, thus revealed that the continuous and fluid lipid membrane, not a layer of immobile adhesive vesicules, was formed. However, the fluorescence signal of the bleached area did not recover to the same level as that before the photobleaching, and we recognized the trace of the photobleaching even after 1200 s as shown in Fig. 1d. This result indicates the existence of immobile fraction in the lipid membrane on the PVC substrate. We checked that the fluorescence signal from PVC substrate was below the background level. Therefore we evaluated the fluidity and the immobile fraction by the quantitative FRAP measurement.

Figure 2 shows the fluorescence recovery curves obtained by the FRAP measurement of the DOPC-lipid membranes containing 0.5% of Rb-DPPE. The recovery plot on the SiO_2/Si substrate asymptotically closed to 1. The FRAP plot of the lipid membrane on the PVC substrate shows incomplete recovery, consistent with the residual photobleached trace observed in Fig. 1d.

On the condition of a pure two-dimensional diffusion of the dye-labeled lipid from an infinite reservoir, the recovery ratio of the fluorescence intensity as a function of time t is given by

$$f(t) = -A \cdot \exp(-2\tau_D/t)[I_0(2\tau_D/t) + I_1(2\tau_D/t) \quad (1)$$

where I_0 and I_1 are the Bessel functions of zero order and first order, respectively, A is the mobile fraction, and $\tau_D = S/4\pi D$ is the characteristic diffusion time, where D is the diffusion coefficient. We fitted the recovery plots to eq (1) and obtained the values of D and A from the best fit between the recovery curves calculated from eq (1) and the experimental data. On the PVC substrate (D = 3.74 μm^2/s, A = 0.68) and the SiO_2/Si substrate (D = 2.03±0.10 μm^2/s, A = 0.97±0.03(n=5)). On the PVC substrate, the mobile fraction of 68% means that 32% of the lipid membrane in the coverage were formed as isolated patches, or trapped by the substrate. It is interesting, however, the mobile fraction showed two-times higher fluidity than that on the SiO_2/Si substrate. It is well known that the lipid bilayer membrane on solid substrates have 2-3 times lower D values than the free-standing lipid bilayers such as giant vesicles [2]. The mobile fraction of the lipid membrane on the PVC substrate may have a similar fluidity to that of a free-standing bilayer.

The details of the microscopic structures of the PVC substrate and the lipid membrane on it will be described.

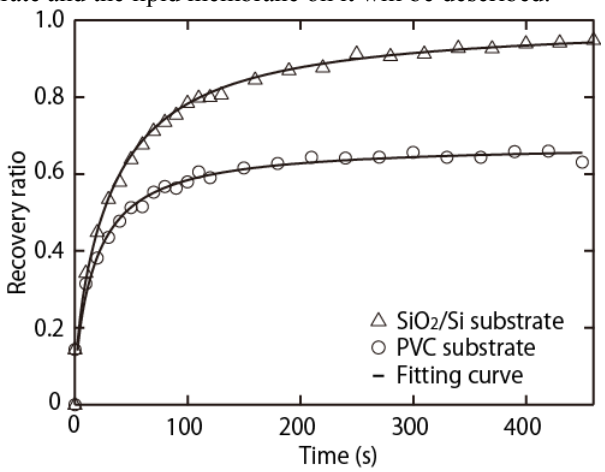

Fig. 2 Temporal fluorescence recovery curves of the DOPC-lipid membranes doped with 0.5% of Rb-DPPE on the PVC substrate, and the thermally oxidized SiO_2/Si substrate. Triangles and circles represent the experimentally obtained values on the SiO_2/Si and PVC substrate, respectively, and the solid lines represent the fitting curve using eq (1).

4. Conclusions

In this study, we formed the DOPC-lipid membranes on the PVC substrate, and evaluated the fluidity of the lipid membrane with experimental methods based on fluorescence microscopy. We prepared the DOPC-lipid membranes with the vesicle fusion method using the sonicated vesicle suspension of DOPC. Fluorescence microscopes gave geometrically uniform images of the lipid membrane on the PVC substrate. The fluidity and the mobile fraction of the lipid membrane was evaluated with FRAP measurement. The mobile fraction in the lipid membrane on the PVC substrate had the diffusion coefficient close to that in a free-standing lipid bilayers, in spite of the existence of the immobile fraction about 30%.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Numbers 23685003, 24760277, 24226010, "Program to Foster Young Researchers in Cutting-Edge Interdisciplinary Research" from JST, and the EIIRIS collaborative project of Toyohashi Tech.

References

[1] T.Hattori, M.Yoshitomo, S.Mori, D.Miyamoto, R.Kato, K.Sawada, *Electroanalysis* 2012, **24**, 114-120.

[2] R.Tero, *Materials* 2012, **5**, 2658-2680

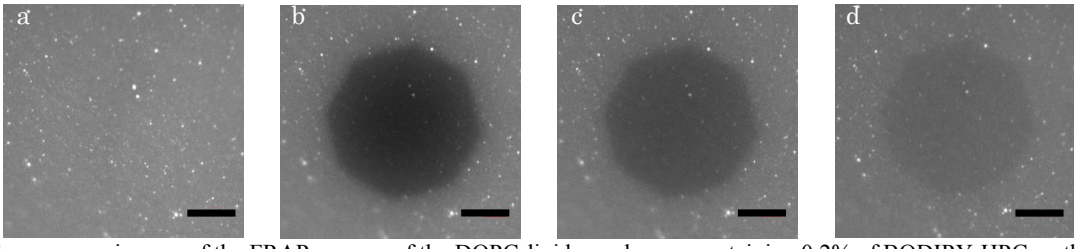

Fig.1 Fluorescence images of the FRAP process of the DOPC-lipid membranes containing 0.2% of BODIPY-HPC on the PVC substrate (scale bar: 20 μm). (a) Before photobleaching, (b) just after photobleaching (t = 0 s), (c) t = 90 s, (d) t = 1200 s.