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Deposition of phospholipid layers on SiO_2 surface modified by alkyl-SAM islands

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Abstract

Formation of the supported planar bilayer of dipalmitoylphosphatidylcholine (DPPC) on SiO_2 surfaces modified with the self-assembled monolayer (SAM) of octadecyltrichlorosilane (OTS) has been investigated by atomic force microscopy (AFM). DPPC was deposited by the fusion of vesicles on SiO_2 surfaces with OTS-SAM islands of different sizes and densities. The DPPC bilayer membrane formed self-organizingly on the SiO_2 surface with small and sparse OTS islands, while did not when the OTS islands were larger and denser. The relative size between the vesicles and the SiO_2 regions is the critical factor for the formation of the DPPC bilayer membrane.

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1. Introduction

Nano-bioelectronics is one of the attractive research fields in these days. Self-assembled monolayers (SAMs) and supported membranes have fascinated enormous attentions as functionalizing methods on inorganic solid surfaces with bio-materials [1–4]. SAMs were applied to the modification of chemical and physical surface properties, for example hydrophilicity and electric charge, and anchoring other molecules such as lipids and proteins in the bio-electronics field [1,2]. Supported membranes have been studied as an active medium of biosensors and bio-mimicking systems of plasma membranes due to the character that membrane proteins can be deposited on solid surfaces remaining the biological functions as well as controlling the orientations [3,4]. Combination of the SAM and the supported membrane techniques brings a big advancement to the development of new biosensors and bio-functionalized devices.

Vesicle fusion (VF) is a feasible and prevalent method to form supported bilayer membranes on solid surfaces [4]. Langmuir–Blodgett (LB) method is another popular technique to fabricate supported membranes [4,5]. However, this method has a definite disadvantage when the deposition to the small (in order of micrometer or less) area is required. It is suitable to the deposition to the homogeneous wide area, since the organic solution of lipids is spread at

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the wide air–water interface. From this view point, VF method seems to be a superior technique for area-selective membrane formations. Membrane structures, formation mechanisms and influences of the vesicle size and cations in the buffer solution in the VF have been studied [6–14]. In these previous studies, however, vesicles were deposited on homogeneous surfaces [6–12] or patterned surfaces in micrometer order [13,14], and no result was reported about the behavior of vesicles on a nano-patterned surface.

In the present study, we have investigated the formation of the lipid bilayer membrane by the VF on co-existing hydrophilic and hydrophobic domains in the order of hundreds of nanometer. Dipalmitoylphosphatidylcholine (DPPC) membranes on the SiO_2 surfaces modified by the SAM islands of octadecyltrichlorosilane (OTS) with varied coverage were observed by means of atomic force microscopy (AFM). It has been found that the membrane formation is highly affected by OTS islands and that the relative size of the lipid vesicles and the SiO_2 regions is the critical factor.

2. Experimental

SiO_2 layers were obtained by thermal oxidation of cleaned Si wafers at 1000 °C, followed by immersion into the H_2O_2/H_2SO_4 solution. The hydrophilicity of the SiO_2 surface was able to be varied depending on the time of H_2O_2/H_2SO_4 treatment. Combination of thermal and chemical oxidation of substrates made it possible to control the size and the density of the OTS islands. OTS-SAM islands were deposited by immersing the SiO_2 substrate in the solution of 10 mM OTS in water saturated toluene for 5 s at RT. The detail of the sample treatment and the OTS deposition will be described elsewhere [15]. Unilamellar vesicles of DPPC were prepared by agitating vacuum-dried DPPC films in the buffer solution (150 mM NaCl, 1.0 mM $CaCl_2$, 10 mM HEPES/NaOH (pH 7.0)) followed by extrusion through a 100 nm polycarbonate filter (Liposo-Fast, Avestin Inc.). The diameter of the 100 nm-filtered DPPC vesicles had been reported to be 125 nm [16]. The suspension was kept above the transition temperature from the gel to the liquid crystal (41 °C for DPPC) during the preparation processes. For the deposition of DPPC membrane, the sample was incubated in the vesicle suspension at 45 °C for 2 h. AFM observations were performed under the buffer solution using an SPI3800 scanning probe microscopy system (Seiko Instruments Inc.) in the dynamic-force mode (tapping mode) using a Si cantilever. When the OTS/SiO_2 surfaces before and after DPPC deposition were observed by AFM, two OTS/SiO_2 pieces, which had been cut out of the identical OTS/SiO_2 sample, with and without DPPC were prepared and successively observed using the same cantilever in order to avoid irregularity of the OTS islands and the tip condition.

3. Results and discussion

Fig. 1 shows AFM images of the OTS/SiO_2 surface measured in the buffer solution before and after the DPPC deposition by the VF method. The total coverage of OTS in Fig. 1a is 0.21. The average diameter of the OTS islands is approximately 322 nm. The space of the SiO_2 regions between the OTS islands is 241 nm on average. Large protrusions are probably polymerized OTS aggregations, which are mainly positioned on the OTS islands. The height of the OTS islands measured in the air was 1.94 nm (data not shown). It is a reasonable value in view of the length of the alkyl chain of OTS (2.5 nm), the tilt of the chain and the compression by the cantilever [1]. It is noted that on a hydrophobic surface, an additional force is applied between the cantilever and the surface due to the solvent exclusion effect in the AFM measurement in an aqueous solution [17]. Therefore the height difference between hydrophilic and hydrophobic domains (SiO_2 and OTS at present) cannot be estimated exactly by AFM in the buffer. In the present experiments, larger values have been obtained for the height of the OTS islands in the buffer than in the air by ~2 nm. After the DPPC deposition, the surface is covered by a flat membrane (Fig. 1b). The second or more layer membranes are not observed. Excess vesicles are almost completely removed during the washing process with the buffer and only the first bilayer membrane remained as previously reported [6,7]. Three regions are observed on the membrane surface in Fig. 1c: dominant brighter (=higher) regions (A), grey (=lower) domains (B) and dark defects (C). The dark defects are intentionally introduced by exposing

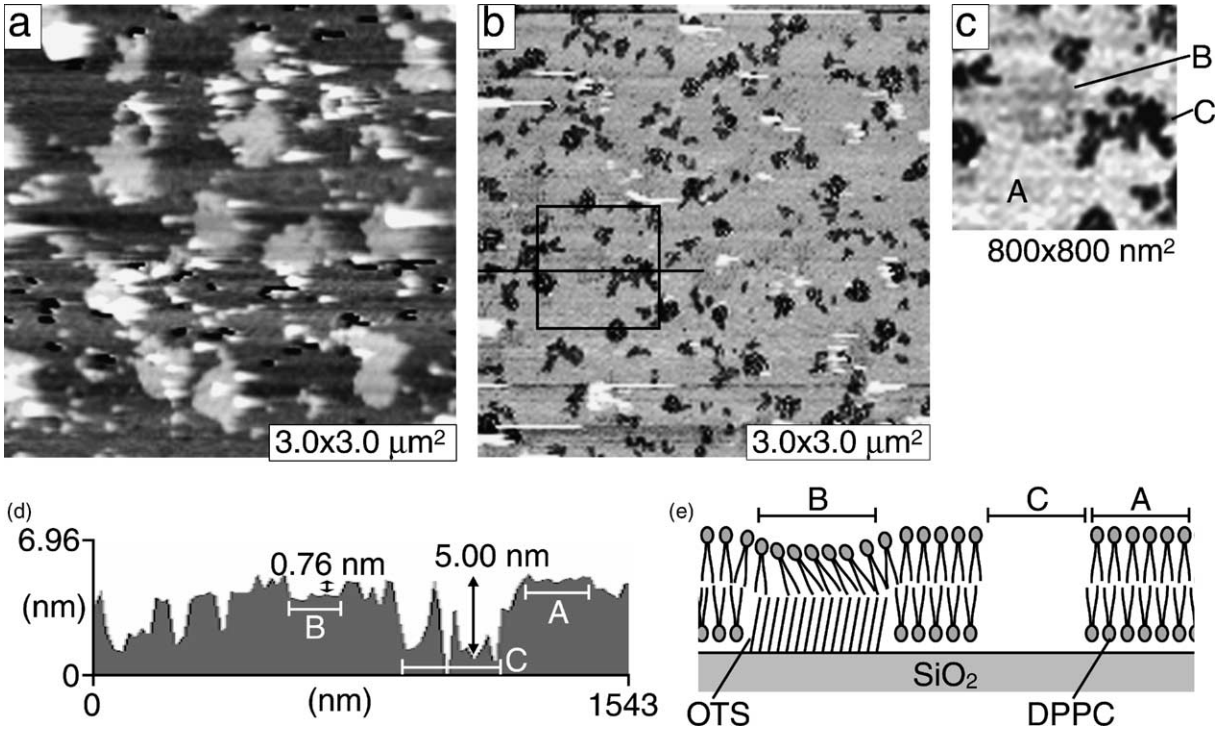

Fig. 1. AFM images ($3.0 \times 3.0\ \mu m^2$) of the OTS-modified SiO_2 surfaces ($\theta_{OTS} = 0.21$) obtained in the buffer solution (a) before and (b) after the deposition of DPPC. (c) The magnified image of the square area in (b) ($800 \times 800\ nm^2$). (d) The profile of the line drawn in (b). (e) Schematic illustration for the line profile in (d).

the deposited membrane surface to the air in a moment. The thickness of the membrane is acquired from the depth of the defect C. The height of the region A measures 5.00 nm (Fig. 1d), which is a reasonable value for a lipid bilayer membrane [7,8]. The domain B, which is lower than the region A by 0.76 nm (Fig. 1d), is assigned to a DPPC/OTS layer from the following reasons. First, the shape and density of the domain B is similar to those of OTS islands before DPPC deposition. Second, almost all the OTS aggregations, which have been positioned on the OTS islands before DPPC deposition (Fig. 1a) are observed in domain B. It is also reasonable from the previous studies, in which the formation of the lipid/SAM bilayer by the VF method has been reported on thiol/Au [9,10,14] and OTS/SiO_2 systems [11]. The value of 0.76 nm as the height difference between the DPPC bilayer (A) and the DPPC/OTS layer (B) seems quite large, because the alkyl chain of OTS ($C_{18}H_{37}$) is longer than the acyl chain of DPPC ($C_{16}H_{31}O$) and tilts only 10° from surface vertical [1]. It is due to the tilted orientation of DPPC on the OTS-SAM (∼36° from the vertical) [11] and the absence of the water layer between the membrane and the substrate. In short, the SiO_2 regions and the OTS islands are covered by the DPPC bilayer and monolayer, respectively, as illustrated in Fig. 1e.

In case that the SiO_2 surface has been pre-modified by OTS islands with high coverage, completely different results are obtained. Fig. 2 shows the AFM images before and after the deposition of DPPC on the SiO_2 surface covered by OTS islands with the coverage of 0.81. The average diameter of the OTS islands is approximately 876 nm and the width of the SiO_2 area between the OTS islands is 83 nm on average. The remarkable difference is that the shape of the OTS islands is clearly observed similarly to Fig. 2a after the deposition of DPPC as shown in Fig. 2b. It indicates that the regular DPPC bilayer membrane does not form on SiO_2 regions between the OTS islands. The

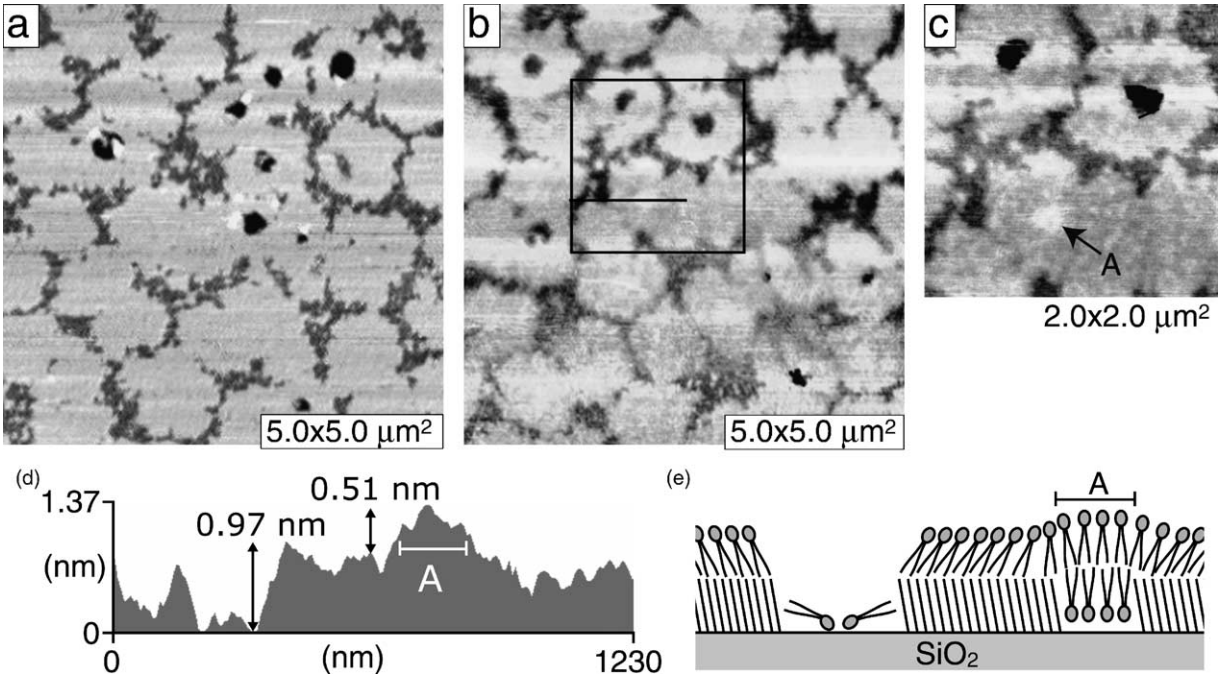

Fig. 2. AFM images ($5.0 \times 5.0\ \mu m^2$) of the OTS-modified SiO_2 surfaces ($\theta_{OTS} = 0.81$) obtained in the buffer solution (a) before and (b) after the deposition of DPPC. (c) The magnified image of the square area in (b) ($2.0 \times 2.0\ \mu m^2$). (d) The profile of the line drawn in (b). (e) Schematic illustration for the line profile in (d). The region "A" represents the DPPC bilayer in the defect of the OTS island observed in (c).

DPPC monolayer would be formed on OTS same as Fig. 1. Circular protrusions (indicated as A in Fig. 2c) higher by 0.51 nm (Fig. 2d) is observed on the DPPC/OTS layer. The number of the protrusions is small, but they are always observed on the DPPC/OTS regions and not on the SiO_2 regions between the OTS islands in several AFM images obtained at different points. They are likely a DPPC bilayer formed in the circular defects observed in Fig. 2a, since the height is close to the height difference between the DPPC bilayer and the DPPC/OTS layer in Fig. 1d. The height difference of the DPPC/OTS domains and the SiO_2 regions is 0.97 nm (Fig. 2d). The value is too small as the height of a DPPC/OTS layer compared with the results in Fig. 1. We consider that a small amount of DPPC has adsorbed on the SiO_2 region with a lying or tilted orientation exposing the acyl chains. The cantilever mainly interacts with hydrophobic acyl chains, that resulted in the artificial height in the AFM image as mentioned above [17]. Even if the actual height difference between the DPPC/OTS and the DPPC on SiO_2 is difficult to estimate, it is certain that the amount of DPPC molecules deposited on the SiO_2 region is too small to form a bilayer. These interpretations are illustrated in Fig. 2e; the DPPC monolayer forms on the OTS islands, but the DPPC bilayer does not on the SiO_2 regions; the DPPC bilayer forms exceptionally in some of the circular defects of the OTS island (indicated as A).

The difference in the formation of DPPC bilayer membranes on SiO_2 region between the cases of Figs. 1 and 2 are explained as follows. In the membrane formation by the VF on a hydrophilic surface, the reaction is carried out through three steps: adhesion, rupture and spreading (Fig. 3a) [8,12]. The point is the adhesion process. Stable adhesion is necessary for the membrane formation. The bilayer membrane is also formed in the same way on the SiO_2 surface modified with OTS islands when wide SiO_2 regions are exposed like Fig. 1. On the other hand, the stable adhesion of vesicles is prevented by OTS islands in case that too narrow SiO_2 regions for vesicles to

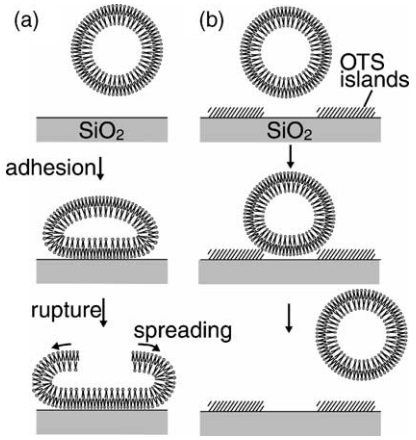

Fig. 3. Schematic illustrations of the mechanism of the membrane formation from the vesicle on SiO_2 surfaces, in the case of (a) bare or sufficiently wide SiO_2 region and (b) a small SiO_2 area on the OTS-modified surface.

adhere are exposed as in Fig. 2 and, as a result, the bilayer membrane does not form. As concerns the formation of a DPPC monolayer on OTS islands, no difference has been observed depending on the island size. It will be because the OTS islands are quite larger than the vesicle size even in Fig. 1. However, the DPPC monolayer on OTS islands may form irrespective of the size of OTS islands, since it is proposed that spreading of lipid monolayer simultaneously proceeded with the adhesion process on a hydrophobic surface [6].

In the previous studies, vesicles have been deposited on homogeneous surfaces [6–12] or sufficiently large areas even on micro-patterned surfaces [13,14]. This is the first study on the VF on the domains with the comparable size to vesicles. The size of the vesicle effective to the membrane formation is in the order of tens to hundreds nanometer [8], which is just the target of the nano-fabrication and patterning. Our results indicate the importance of controlling the relative size of vesicles and surface domains.

4. Conclusion

In the present study, we have investigated the formation of supported bilayer membrane of DPPC on SiO_2 surfaces modified with OTS-SAM islands by AFM. The DPPC bilayer formation on the bare SiO_2 region was considerably affected by the size and densities of the OTS islands; the bilayer membrane formed on SiO_2 with small and sparse OTS islands, but did not with large and dense OTS islands. We regard the critical factor as the relative size between the vesicle and the SiO_2 region. In case that the size of the SiO_2 region is decreased to comparable with the vesicle size, the adhesion of the vesicles is prevented by hydrophobic OTS islands.

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