Effect of Chemical Modification of the Substrate Surface on Supported Lipid Bilayer Formation

Toshinari Isono,* Hanako Tanaka, and Toshio Ogino
Graduate School of Engineering, Yokohama National University,
Tokiwadai 79-5, Hodogaya, Yokohama 240-8501, Japan
(Received 21 February 2007; Accepted 1 May 2007; Published 10 May 2007)

We have investigated the effect of chemical state control of the Si substrate surfaces for the formation of low-defective uniform supported lipid bilayers. To form supported lipid bilayers, we used the vesicle fusion method. In the bilayer formation processes, control of the interfaces between the vesicles and the surfaces is important. To examine the effect of the oxide formation process on Si surfaces, we used SiO$_2$ surfaces prepared by chemical acid-treatment and those by thermal oxidation. SiO$_2$ surfaces are generally hydrophilic. To change the chemical state of the SiO$_2$ surfaces, they were modified with various self-assembled monolayers. These surfaces were immersed in a solution with lipid vesicles suspension, and the lipid vesicles were transformed into planar bilayers. The hydrophilic surface is more suitable for the uniform lipid bilayer formation than the hydrophobic surface. The bilayer coverage on the thermal oxide surface is larger than that on the chemical oxide one. These results indicate that the surface chemical states influence the efficiency of the supported lipid bilayer formation. [DOI: 10.1380/ejssnt.2007.99]

Keywords: Biophysics, medical physics, and biomedical engineering; Self-assembly; Surface chemical reaction; Surface energy; Water; Supported lipid bilayer; Vesicle fusion method; Biointerface

I. INTRODUCTION

Bio-compatible surfaces and interfaces are required in the functional analysis of living cells and biological molecules as well as in the application of the solid surfaces to various biotechnological devices because those cells and molecules have to be immobilized on the surfaces without denaturation of the bioactivities. To fabricate the bio-compatible interfaces, control of initial surfaces of the substrates is crucial.

A cell surface consists of a lipid bilayer and membrane proteins. The research on membrane proteins is important because the functional analyses of the membrane proteins are closely related to the biology and creation of new drugs. Artificial supported lipid bilayers on solid surfaces are often used as a model system of the cell membranes in in vitro studies on the fundamental properties of the membrane proteins [1].

To form supported lipid bilayers and monolayers, we used the vesicle fusion method illustrated in Fig. 1 [2–5]. Supported lipid bilayers, which consist of two amphiphilic phospholipid layers facing to each other, are formed by fusion and rupture of the vesicles. In the bilayer formation process, hydrophilic and/or hydrophobic interactions between the vesicles and the substrate surfaces are essential because the vesicle surfaces are accompanied with low-mobility or bound water molecules. Generally the surface control includes structural and chemical approaches. In the formation of low-defective uniform bilayers, the chemical state control plays a more important role [6]. We focused on the chemical controls of the substrate surfaces, in particular control of their hydrophilicity or hydrophobicity. We also examined the effect of the surface charge by modifying the surfaces with various self-assembled monolayers (SAMs) [7].

II. EXPERIMENTS

The Si (001) substrates were cleaned by a mixture of concentrated H$_2$SO$_4$ and 30% H$_2$O$_2$ (volume ratio of 3:1), and then the surface oxides were removed by a diluted 5% HF solution. To examine the effect of the oxide formation process, the surfaces were chemically oxidized using a mixture of 35% HCl, 30% H$_2$O$_2$ and H$_2$O (1:1:4) or thermally oxidized at 800°C for 30 min in air. SiO$_2$ surfaces are generally hydrophilic. To make SiO$_2$ surfaces hydrophobic, octadecyltrichlorosilane (OTS) was deposited on the SiO$_2$ surfaces [8]. To form cationic surfaces, the SiO$_2$ surfaces were modified with 3-aminopropyltriethoxysilane (APTES) [9]. Since the APTES surfaces are terminated with the amino groups, the APTES surfaces have positive charge. For the anionic surfaces, 2-(carbomethoxy)ethyltrichlorosilane (CMETS)

*Corresponding author: isono@pc5.oginolab.dnj.ynu.ac.jp

FIG. 1: Schematics of (a) lipid bilayer formation process by the vesicle fusion method on the hydrophilic surface, and (b) lipid monolayer formation process on the hydrophobic surface.
was used and the CMETS-deposited surfaces were treated by 70% HNO₃ for hydrolysis [10]. Because the CMETS surfaces are terminated with the carboxyl groups after hydrolysis, the acid-treated CMETS surfaces have negative charge. All the SAMs were prepared by the dipping method. First, the OTS, APTES, and CMETS solutions were diluted in dehydrated toluene (0.625 vol.% for OTS and APTES, and 0.175 vol.% for CMETS, respectively). Then the solutions were kept for 8 min at room temperature for OTS, and APTES, and for 30 min at -10°C for CMETS, respectively. In this step, two dimensional planer networks were self-assembled from SAM monomer molecules. Second, the SiO₂ surfaces were immersed in those solution (OTS, APTES: for 5 min at room temperature, CMETS: for 2 hour at -10°C). In this method, the SAMs were deposited on the SiO₂ surfaces.

The lipid bilayers were deposited by the vesicle fusion method on the oxidized surfaces and the chemically modified surfaces. The materials to form the lipid bilayers are 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC) and fluorescently labeled 1-Myristoyl-2-[12-(7-nitro-2-1,3-benzoxadiazol-4-yl)aminododecanoyl]-sn-Glycero-3-Phosphocholine (NBD PC). The mixture of the lipids (DMPC : NBD PC = 100 : 1 w/w) was dissolved in chloroform. The lipid solution was vacuum-dried to remove the solvent and lipid thin films are obtained. The lipid films were added to a buffer solution (150mM KCl, 1.0mM CaCl₂, 10mM HEPES/NaOH, pH 7.4) warmed up above the gel-liquid crystal transition temperature (23.9°C for DMPC). The vesicles were assembled by stirring this lipid solution. We prepared large unilamellar vesicles (LUVs) by sonication using a bath-type sonicator [11]. To facilitate the vesicle rupture on the substrate surface, the lipid solution temperature was kept above the phase transition temperature (at 50°C) during the incubation for 60 min. The formed lipid bilayers were observed by a fluorescence microscopy (OLYMPUS BX51) in a buffer solution.

III. RESULTS AND DISCUSSION

Figure 2 shows fluorescence microscope images of the supported lipid bilayers deposited on (a) a hydrogen-terminated surface, (b) a chemically oxidized surface, and (c) a thermal oxide surface. In the fluorescence microscopy, a lipid bilayer including fluorescently labeled lipid molecules is observed as a bright island. Bright spots are unruptured vesicles. On the hydrogen-terminated surface, neither lipid bilayer nor monolayer formed. Only unruptured vesicles were observed. Lipid bilayer islands, on the other hand, formed on the oxidized surfaces and the bilayer coverage was much larger on the thermally oxidized surface than the chemically oxidized one. The lipid bilayers formed uniformly on the thermally oxidized surface and the bilayer coverage was more than 0.5. The lipid bilayers formed also on the chemically oxidized surface, but bilayer formation was nonuniform and the coverage was less than 0.1.

The above results show that the hydrophilic surfaces are more suitable for uniform supported lipid bilayer formation than the hydrophobic surfaces. Generally, a hydrophilic surface is covered with water molecules of about one monolayer [12]. The mobility of the bound water molecules is much smaller than that of the bulk water molecules. Therefore, the interface between the hydrophilic surface and the vesicle surface should be considered as the interface between the bound water layer and the vesicle surface. The vesicles are hydrophilic and accompanied with the bound water layers on their surfaces. On the hydrophilic surface, the interface structure consists of four layers: the vesicle surface, the water layer bound to the vesicles, the water layer bound to the sub-
strate surface, and the substrate surface in a buffer solution. The interface energy between the substrate surface with the bound water layer and the vesicles surface with the bound water layer is small. Therefore the vesicle can deform to increase the interface area on the surface and rupture by its large curvature around the edge. On the hydrophobic surface, on the other hand, the interface is a three-layer structure consisting of the vesicle surface, the water layer bound to the vesicles, and the substrate surface in a buffer solution. The interface energy between the hydrophobic substrate surface and the water layer bound to the vesicles is large. Because the large interface energy reduces the interface area, the vesicle keeps its spherical shape. In this case, the vesicle does not rupture.

The bilayer islands on the thermally oxidized surface were more uniform than that on the chemically oxidized surface. The density of OH group on the thermally oxidized surface is smaller than that on the chemically oxidized surface because surface OH groups are dehydrated. The surface OH groups strongly attract water molecules by hydrogen bonding. Therefore the oxide surface is accompanied with the bound water layer. The chemically oxidized surface has a high-density OH groups and, therefore, a high-density of water molecules is bound to the surface. The interface energy between two bound water layers accompanied with the vesicles and the substrate surface can be regarded as a water/water interface. Therefore, the bilayer coverage on the thermally oxidized surface is small. On the other hand, because the surface OH group density is low on the thermally oxidized surface, the bulk water or the water bound to the vesicles is attracted to the hydrophilic surface. Therefore, the vesicles are attached to the substrate surface. Once the vesicles are captured on the surface, they deform and finally rupture to form bilayers.

Figure 3 shows fluorescence microscope images of the lipid bilayers on the OTS-, APTES-, and the acid-treated CMETS-modified surfaces. No lipid bilayer and no monolayer formed on the OTS surface. On the APTES and acid-treated CMETS surfaces, on the other hand, lipid bilayers were observed.

When small unilamellar vesicles (SUVs) are used, lipid monolayer can form on the alkyl-SAM treated surface in spite of the high interface energy between the vesicles and the surface as illustrated in Fig. 1 [8, 13]. Because the SUVs have large curvature in the contact to the surface, they rupture at a high rate than the LUVs. In this work, we used the LUVs and no lipid monolayer formed on the OTS surface.

The effect of the surface charge was found to be small. Lipid bilayer coverage is almost same between the cationic surface and the anionic surface. Because the phospholipids used in the experiments are neutral, the interaction through the surface charge is less important. The difference in the oxide formation process is experimentally small when the surface is modified with the SAMs. This indicates that the properties of the SAM surface are independent of properties of the oxidized surface before the SAM deposition.

IV. SUMMARY

We have studied effects of surface modification of the substrate on the formation of supported lipid bilayers using the vesicle fusion method. The most important factor for the uniform lipid bilayer formation is control of hydrophilic/hydrophobic property of the surface. The surface charge is less important in the case of a neutral lipid. When the vesicles are deposited on the Si oxide surface directly, the oxide formation process influences the efficiency of the lipid bilayer formation. When the oxidized surfaces were coated with various SAMs, the oxide formation process did not influence the vesicle fusion process.
Acknowledgments

This work was partly supported by CREST/JST and Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology. The authors would like to thank Prof. Urisu, Dr. Tero and Dr. Oya for their support.

[1] T. V. Ratto, and M. L. Longo, Biophys. J. 83, 3380 (2002).
[2] J. Jass, T. Tjärhage, and G. Puu, Biophys. J. 79, 3153 (2000).
[3] R. P. Richter, R. Bérat, and A. R. Brisson, Langmuir 22, 3497 (2006).
[4] F. F. Rossetti, M. Textor, and I. Reviakin, Langmuir 22, 3467 (2002).
[5] A. T. A. Jenkins, R. J. Bushby, S. D. Evans, W. Knoll, A. Offenhäusser, and S. D. Ogier, Langmuir 18, 3176 (2002).
[6] A. M. Brozell, M. A. Muha, B. Sanii, and A. N. Parikh, J. Am. Chem. Soc. 128, 62 (2006).
[7] V. Atanasov, N. Knorr, R. S. Duran, S. Ingebrandt, A. Offenhäusser, W. Knoll, and I. Köper, Biophys. J. 89, 1780 (2005).
[8] R. Tero, M. Takizawa, Y.-j. Li, M. Yamazaki, and T. Urisu, Langmuir 20, 7526 (2004).
[9] Y.-H. Kim, M. M. Rahman, Z.-L. Zhang, N. Misawa, R. Tero, and T. Urisu, Chem. Phys. Lett. 420, 569 (2006).
[10] R. Tero, N. Misawa, H. Watanabe, S. Yamamura, S. Nambu, Y. Nonogaki, and T. Urisu, e-J. Surf. Sci. Nanotech. 3, 237 (2005).
[11] R. A.-Hamdah, W.-J. Cho, S.-J. Cho, A. Jeremic, M. Kelly, A. Elena, and B. P. Jena, Cell Biology International 28, 7 (2004).
[12] S. M. Bhattacharyya, Z.-G. Wang, and A. H. Zewail, J. Phys. Chem. B 107, 13218 (2003).
[13] C. A. Keller, and B. Kasemo, Biophys. J. 75, 1397 (1998).