Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Patterning Process of Membrane-Associated Proteins on a Solid Support with Geometrical Grooves

Cherlhyun Jeong [a], Tae-Young Yoon [a], Sin-Doo Lee [a], You-Gyoung Park [b] & Hoonjeong Kwon [b]

[a] School of Electrical Engineering, Seoul National University, Kwanak, Seoul, Korea

[b] Department of Food and Nutrition, Seoul National University, Seoul, Korea

Version of record first published: 31 Jan 2007

To cite this article: Cherlhyun Jeong, Tae-Young Yoon, Sin-Doo Lee, You-Gyoung Park & Hoonjeong Kwon (2005): Patterning Process of Membrane-Associated Proteins on a Solid Support with Geometrical Grooves, Molecular Crystals and Liquid Crystals, 434:1, 297/[625]-303/[631]

To link to this article: http://dx.doi.org/10.1080/15421400590955046

Mol. Cryst. Liq. Cryst., Vol. 434, pp. 297/[625]–303/[631], 2005

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400590955046

Patterning Process of Membrane-Associated Proteins on a Solid Support with Geometrical Grooves

Cherlhyun Jeong
Tae-Young Yoon
Sin-Doo Lee
School of Electrical Engineering, Seoul National University, Kwanak, Seoul, Korea

You-Gyoung Park
Hoonjeong Kwon
Department of Food and Nutrition, Seoul National University, Seoul, Korea

We have shown a patterning process of membrane-associated proteins through spontaneous assembling of the lipid anchors on a solid support with geometrical grooves. The lipid anchors possessing unbalanced effective molecular shapes are assembled near geometrical groove structures so as to minimize the free energy of elastic distortions. The specific patterning of the lipid anchors and the membrane-associated proteins can be attained without disturbing two-dimensional lateral fluidity of a supported membrane. Our patterning concept of the supported membrane would be applicable for devising biosensors and protein chips.

Keywords: biosensors; lipid bilayer; membrane-associated protein; supported membrane; surface patterning

INTRODUCTION

Biochemical functions of membrane-associated proteins, such as bacterial toxins, are typically regulated by specific binding to membrane receptors. For example, extensive studies have been carried

This work was supported in part by Center for Electro- and Photo-Responsive Molecules through Korea University.

Address correspondence to Sin-Doo Lee, School of Electrical Engineering, Seoul National University, Kwanak P.O. Box 34, Seoul 151-600, Korea. E-mail: sidlee@plaza.snu.ac.kr

out for cholera toxins which bind to their lipid anchors in the plasma membrane, monosialoganglioside (GM1) [1]. Especially, supported lipid membranes that produce plasma membrane-mimetic circumstances *in vitro* have provided a wealth of information on these bacterial toxin-membrane receptor interactions [2]. Such utility of the supported membrane is predominantly attributed to two-dimensional fluidity of lipid molecules retained in supported membranes [3]. Furthermore, it has been recently reported that supported membranes can be patterned, for example, using a chemical patterning of solid supports [4,5], which provides more versatile opportunities to study the membrane associated protein interactions *in vitro*.

However, existing patterning technologies prohibit lateral diffusion of all the lipid components across diffusion barriers, thereby restricting lipid-lipid or lipid-protein interactions within a small domain that is bounded by diffusion barriers. Therefore, a new patterning technology, which is specific to particular types of lipids without disturbing two-dimensional fluidity of supported membranes [6], is needed. We developed such patterning technology which is used for creating elastic distortions in supported membranes. Because of the elastic distortions, various lipids components tend to redistribute according to their effective molecular shapes to relax the elastic strain. In this work, we demonstrate the spontaneous assembling process of the GM1 at predetermined sites due to geometrical energy barriers based on our patterning concept. The assembling process of the GM1 leads directly to a concentrated binding of cholera toxins at these sites, inevitably necessary for both the biological studies and the *in vitro* applications such as protein chips.

BASIC CONCEPT

The basic concept of our patterning technology is illustrated in Figure 1, in which geometrical grooves are formed on the solid support. Because of the small persistence length of fluid lipid membranes (of order of ten nanometers), the supported membrane closely follows the morphology of geometrical grooves [7] to minimize the free energy of elastic distortions created around geometrical grooves.

When the supported membrane consists of lipids with different effective molecular shapes (EMS's), there should exist a stable state in which the elastic free energy is minimized [6]. For example, assume that one type of constituting lipids has a balanced EMS, e.g., dioleoyl(18:1)-phosphatidylcholine (DOPC, Fig. 1(a)), while the other type has an unbalanced EMS, e.g., GM1 (Fig. 1(b)) of which head group (containing anchors for membrane-associated proteins) is large

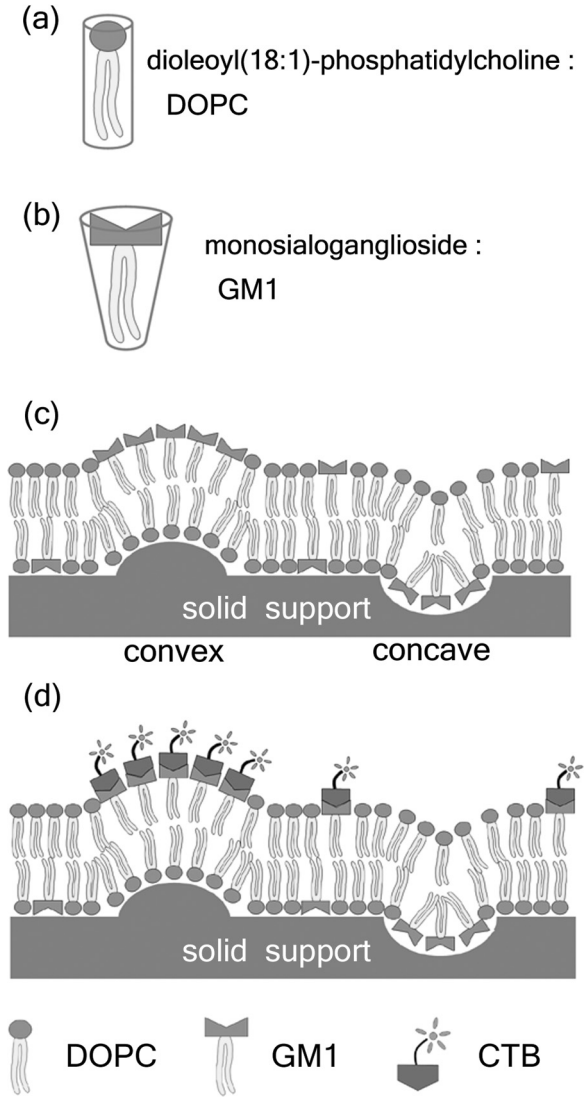

FIGURE 1 The lipid molecules with (a) a balanced and (b) an unbalanced (a large head group area) effective molecular shapes. (c) The spontaneous assembling of the lipids with an unbalanced effective molecular shape, at the geometrical groove with a positive spontaneous curvature. (d) The concentrated binding of cholera toxin subunit-B (CTB) with the GM1 at the geometrical groove.

compared to the hydrocarbon volume. In this case, the GM1 in the outer leaflet of the supported membrane will be assembled at the geometrical groove with the convex shape to fit its EMS into the geometrical groove (Fig. 1(c)). The spontaneous assembling process of the GM1 obviously results in a concentrated binding of cholera toxins (Fig. 1(d)). Therefore, the presence of convex structures in the solid support produces preferential binding sites of cholera toxins subunit-B (CTB) in the supported membrane.

MATERIALS AND METHODS

Supported bilayers were formed using a vesicle fusion method [8,9]. A solid support was prepared by depositing a SiO_2 layer of 1.5 μm thick on top of a quartz wafer which suppresses any undesired interference [10] by the small difference in the refractive indices between the SiO_2 and the quartz (less than 0.08). Geometrical structures were made by scratching the solid support using a diamond cutter with a typical width of 20 μm (Fig. 2(b)).

In order to realize our concept described above, the constituting lipids should have different EMS's. A binary mixture of a lipid with a balanced EMS, the DOPC (Avanti, Birmingham, AL) with a slightly negative spontaneous curvature (-0.00625 Å^{-1}) [11], and a lipid with unbalanced EMS, the GM1 (Avanti, Birmingham, AL) with positive spontaneous curvature, was used to produce the unilamellar vesicles. The DOPC and the GM1 were dissolved in chloroform and a solution of 2:1 chloroform/methanol before use, respectively, with the molar ratio of 99:1. Using a stream of the nitrogen gas, the solvent was evaporated. The sample was desiccated under a reduced pressure at least for 5 hours to remove any residual chloroform and methanol. The vesicle solutions were prepared by hydration of a dried lipids in Tris buffer (100 mM NaCl, 10 mM Tris · HCl, distilled water at pH 8.0) with a total lipid concentration of 0.2 mg/ml. After the hydration, the small unilamellar vesicles (SUVs) were obtained using the extrusion method (Mini-Extruder, Avanti) with over 60 filtering processes through a carbon-filter of 100 nm pores at room temperature. The supported membrane was formed through the fusion of SUV on the solid support for 10 minutes at room temperature.

After the bilayer deposition, the samples were thoroughly rinsed by distilled water to remove any unfused SUVs from the lipid bilayer surfaces. The CTB (Molecular Probes, Eugene, OR) conjugated to Alexa-488 had dissolved in phosphate buffered saline (PBS) solution (0.05% sodium azide at pH 7.2) in concentration of 0.1 mg/mL and stored at 4°C the before CTB solutions were applied to the supported

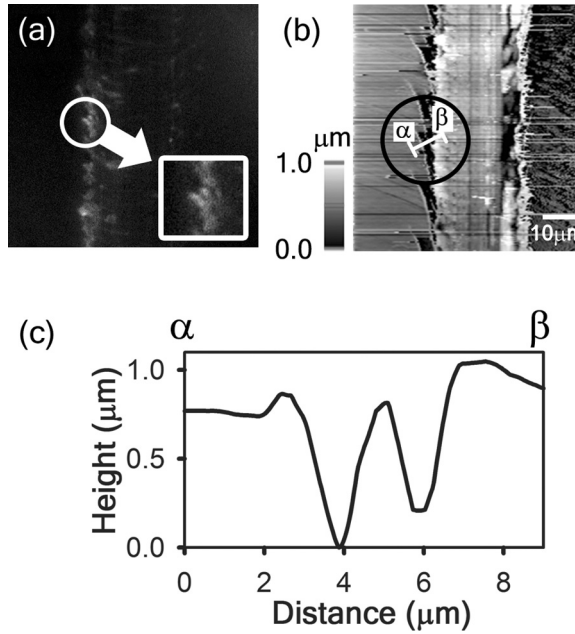

FIGURE 2 (a) The fluorescence microscope image of the supported membrane formed on the geometrical scratch of the solid supports. (b) The morphology of the geometrical scratch observed by the atomic force microscopy. (c) The line profile of the geometrical scratch from the position α to the position β inside black circle in (b).

membrane. After 10 min incubation, the surface was thoroughly rinsed again by distilled water.

RESULTS AND DISCUSSION

Figure 2(a) is the fluorescence microscope image of the CTB bound in a supported membrane formed on the geometrical scratch. By comparing Figure 2(a)–2(b) showing the morphology of the scratch observed by the atomic force microscope (AFM), the concentrated CTB binding with the GM1 is clearly shown at the geometrical structure. Because of the specific binding property of the GM1 with positive spontaneous curvature, the GM1 are spontaneously assembled at the geometrical groove. In the white square region in Figure 2(a), the bright and dark images indicate the assembling characteristics of the GM1. Figure 2(c) is a line profile of the geometrical scratch from the position α to the position β inside the black circle in Figure 2(b). The bright and dark

areas in a white square in Figure 2(a) correspond to hills and valleys shown in Figure 2(c), respectively. Because the CTB bind with the GM1 after the membrane formation, the CTB-GM1 binding pairs mainly appear in the outer leaflet of the lipid bilayer on the solid supports. Therefore, the concentrated binding of the CTB in a convex shape i.e., at the hills of Figure 2(c) clearly supports that the spontaneous assembling of lipids arises from the curvature elasticity. More specifically, the spontaneous assembling process is originated from the relaxation of the elastic distortion energy generated by the geometrical grooves.

One interesting point is that the spontaneous assembling process is associated with in the lateral fluidity. If there is no lateral fluidity, the redistribution of constituting lipids is prohibited, and thus no assembling process takes place. We now examine the lateral fluidity in the presence of the spontaneous assembling process using the conventional electrophoresis [5] indirectly. Instead of the CTB-GM1 binding pair which has low mobility and an uncharged head group, a charged lipid having the similar property of the EMS, Texas Red-dihexadecanoyl-phosphoethanolamine (Texas Red-DHPE, Molecular Probes, Eugene, OR) is used for studying the lateral fluidity. Since the Texas-Red DHPE carries a net single negative charge while the DOPC has no net charge, the Texas-Red DHPE drifts in the plane of the supported membrane when an electric field is applied parallel to the membrane. Figure 3 shows the fluorescence microscopic image for electrophoresis, which was taken in 1 hour after the electric field of 10 V/cm was applied. The fluorescence is found to remain uniform around the assembling site in contrast to the diffusion barrier (see the arrows in Fig. 3) created purposely by scraping off the supported membrane with a diamond cutter. Since such diffusion barrier completely separates the supported membrane, the diffusion barrier sets up a steady-state fluorescence gradient profile. Therefore, the uniform fluorescence around the assembling site provides a definite evidence of both continuity and the lateral fluidity of the supported membrane is preserved across the assembling site.

CONCLUSION

We have shown the spontaneous assembling process of the GM1 at specific groove structures and the resultant concentrated binding of cholera toxins at those predetermined sites. The assembling process arises from the relaxation of the elastic free energy by the redistribution of lipids to fit its curvature into the geometrical grooves. Furthermore, this pattering process in a supported membrane is

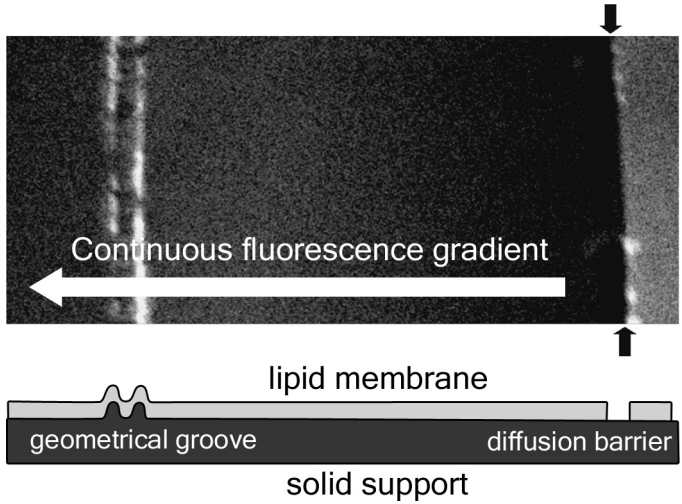

FIGURE 3 The fluorescence microscope image of the supported membrane under the electric field of 10 V/cm applied parallel to the membrane for 1 hour. The uniform fluorescence around the spontaneous assembling site provides an evidence of both the continuity and the lateral fluidity across the geometrical scratch.

achieved without affecting two-dimensional lateral fluidity. Considering that membrane receptors or lipid anchors produce unbalanced EMS's, our patterning technology of supported membrane would be useful for protein chips.

REFERENCES

[1] Song, X., Shi, J., & Swanson, B. (2000). *Anal. Biochem.*, *228*, 35.
[2] Sackmann, E. (1996). *Science*, *271*, 43.
[3] Boxer, S. G. (2000). *Curr. Opin. Chem. Biol.*, *4*, 704
[4] Groves, J. T. & Boxer, S. G. (2002). *Acc. Chem. Res.*, *35*, 149.
[5] Groves, J. T., Ulman, N., & Boxer, S. G. (1997). *Science*, *275*, 651.
[6] Yoon, T.-Y., Jeong, C., Kim, J. H., Choi, M. C., Kim, M. W., & Lee, S.-D. *Appl. Surf. Sci.*, accepted for publication.
[7] Swain, P. S. & Andelman, D. (2001). *Phys. Rev., E 63*, 051911.
[8] Reviakine, I. & Brisson, A. (2000). *Langmuir*, *16*, 1806.
[9] Keller, C. A., Glasmästar, K., Zhdanov, V. P., & Kasemo, B. (2000). *Phys. Rev. Lett.*, *84*, 5443.
[10] Braun, D. & Fromherz, P. (1998). *Phys. Rev. Lett.*, *81*, 5241.
[11] Chen, Z. & Rand, R. P. (1997). *Biophys. J.*, *73*, 267.