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Amphiphobic Septa Enhance the Mechanical Stability of Bilayer Lipid Membranes

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Abstract

Bilayer lipid membranes (BLMs) provide well-defined systems for screening effects of drugs that act on ion channels. However, the application of this technique is limited due to the low stability of BLMs and low reconstitution efficiency of ion channels into BLMs. Previously, we succeeded in improving the stability of BLMs based on the fabrication of microapertures with a nano-tapered edge in SiO_2/Si_3N_4 septa and efficiently incorporating ion channels using a centrifugal force. Although BLM stability and incorporation probability were dramatically improved by these approaches, some BLMs ruptured when subjected to a centrifugal force. To further improve the BLM stability, we explored the effect of surface modification of the septa on the BLM stability. The modified surfaces were characterized in terms of hydrophobicity, lipophobicity and surface roughness. Diffusion coefficients of lipid monolayers formed on the modified surfaces were also measured. Highly fluidic lipid monolayers were formed on amphiphobic substrates that had been modified with long-chain perfluorocarbons. BLMs formed in amphiphobic septa showed a much higher mechanical stability than those formed in septa that had been modified with a short alkyl chain. These results show that highly stable BLMs can be formed when the surface of the septa has amphiphobic properties. This approach to improve the BLM stability increases the experimental efficiency of the BLM system and will contribute to the realization of high-throughput drug screening platform for ion channels.

1. Introduction

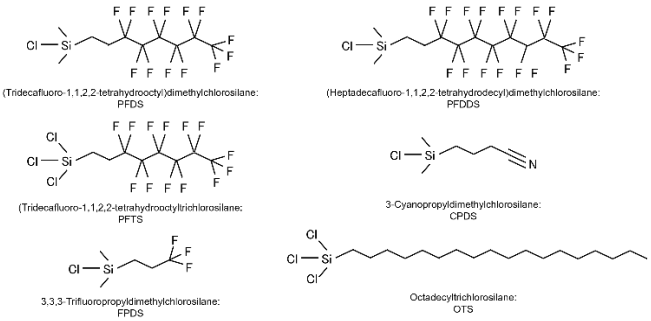
The cell membrane is composed of a bilayer lipid membrane (BLM), which consists of two lipid monolayers facing each other, and various membrane proteins. Among them, an ion channel protein plays a crucial role in transmembrane signaling and is a major target for drug design. Reconstituting ion channels into artificially formed BLMs provides a well-defined system for screening effects of drugs that act on them. However, instability of the BLM system and low efficiency of channel incorporation hinder the widespread application of the BLM systems. We previously addressed these drawbacks by fabricating a nano-tapered microaperture in SiO_2/Si_3N_4 septa [1] and using a centrifugal force [2]. Stable BLMs were successfully formed in the nano-tapered microapertures, and application of a centrifugal force during proteoliposome fusion dramatically improved incorporation efficiency. However, some BLMs ruptured under the centrifugal force. Therefore, further enhancement in BLM stability would be necessary. In the present study, we investigated effects of modifying the surface of the SiO_2/Si_3N_4 septa on the stability of BLMs formed in the septa. The applicability of our approach as the drug-screening platforms for ion channel is also discussed.

2. Experimental

$SiO_2/Si_3N_4/Si$ substrates were immersed in 2% (v/v) solutions of silane coupling agents (OTS, PFDS, PFDDS, PFTS, CPDS and FPDS, Fig. 1) for 6 h in a nitrogen-filled glove box. The contact angles of pure water and oil (n-hexadecane) on each modified substrate were measured by the sessile droplet method. The root-mean-square (RMS) surface roughness for each modified surface was measured by tapping mode atomic force microscopy (AFM). Lipid monolayers were formed on the modified substrates by the vesicle fusion method. The diffusion coefficient of the lipid monolayer was evaluated by fluorescence recovery after photobleaching (FRAP).

Microapertures were fabricated in SiO_2/Si_3N_4 septa according to the procedure described in ref. [3]. BLMs were formed in the microaperture by the folding method and their stability was evaluated in terms of tolerance to an applied centrifugal force with and without proteoliposomes and tolerance to movement of waters surrounding the BLMs.

Fig. 1 Structure of silane coupling agents used in the present study.



3. Results and discussion

We first performed surface characterization of each modified substrate and determined diffusion coefficient of lipid membranes on the modified substrates (Table I). The water contact

Table I. Surface characterization of modified substrates and diffusion coefficients of lipid monolayers formed on the substrates

surface modification	water contact angle [degree]	*n*-hexadecane contact angle [degree]	surface roughness RMS [nm]	diffusion coefficient [μm²/s]
PFDS	107± 2	63 ± 2	0.47 ± 0.22	1.74 ± 0.43
OTS	113 ± 1	36 ± 1	0.39 ± 0.10	1.50 ± 0.45[b]
PFDDS	109 ± 1	65 ± 3	0.65 ± 0.15	1.48 ± 0.28
FPDS	90 ± 1	40 ± 1	0.53 ± 0.11	0.94 ± 0.16
PFTS	116 ± 1	78 ± 1	1.30 ± 0.02	0.92 ± 0.13
CPDS	74 ± 1	12 ± 1	0.36 ± 0.11	0.67 ± 0.28

angles for substrates that were modified with silanes which-have a long perfluoro chain (PFDS, PFDDS, PFTS) or a long hydrocarbon chain (OTS) were larger than 90º, whereas the substrate modified with a silane having a short hydrocarbon chain and a polarized head group (CPDS) showed a smaller water contact angle, indicating that the CPDS-modified substrates were less hydrophobic. On the other hand, oil contact angles larger than 60º were observed on substrates that were modified with PFDS, PFDDS and PFTS, while smaller oil contact angles were observed on OTS- or CPDS-modified substrates. All of the modified surfaces with the exception of PFTS showed a surface roughness smaller than 0.7 nm. Lipid monolayers formed on the PFDS- and PFDDCS-modified surface, which showed large water and oil contact angles, showed high diffusion coefficient. On the other hand, on the FPDS- and CPDS-modified surfaces, which showed smaller water and oil contact angles, the lipid monolayers exhibited much lower diffusion coefficients. These results suggest that a lower interaction between lipids and underlying surfaces allows lipid molecules to glide more rapidly over the surface. Although, the PFTS treatment also resulted in the formation of an amphiphobic surface, the lipid monolayers formed on PFTS-modified surfaces had less fluid characteristics than those on the PFDS- and PFDDS-modified surfaces, possibly because the large surface roughness interferes with the diffusion of lipid molecules.

To examine the effect of surface modification of the chips on the stability of BLMs, we compared the stability of BLMs formed on PFDS- and CPDS-modified Si chips since the lipid monolayers formed on these surfaces showed the largest difference in lipid fluidity. The stability of the BLMs were evaluated by measuring the tolerance to movement of the solutions surrounding BLMs, and the tolerance to centrifugal force (CF). It was found that 75% of the BLMs formed on PFDS-modified chips showed a membrane resistance in excess of 1 GΩ, even after 20 aspiration cycles (ACs) (Table II). On the other hand, only 50% of the BLMs formed on CPDS-modified chips survived the same treatment. We also compared the tolerance of BLMs to a CF with and without proteoliposomes. As shown in Table II, when the BLMs were centrifuged at 55 × g in the absence of proteoliposomes, all of the BLMs on the PFDS-modified chips survived this treatment. In contrast, half of the BLMs on the CPDS-modified chips were broken during centrifugation. In the presence of proteoliposomes, decreased survival rates of 71 % and 43 % were observed for the BLMs formed on PFDS- and CPDS-modified chips, respectively, even if lower CF of 40 × g was

Table II. Formation probability, lifetime, and background current noise level of the BLMs suspended on modified Si chips

Surface modification	tolerance to ACs	tolerance to CF without proteoliposomes	tolerance to CF with proteoliposomes
PFDS	75% (9/12)	100% (11/11)	71% (12/17)
CPDS	50% (3/6)	50% (5/10)	43% (3/7)

applied. Thus, the mechanical stability of the BLMs formed on the PFDS-modified chips was clearly superior to those formed on the CPDS-modified chips. These results confirm that the mechanical stability of free-standing BLMs is strongly correlated with the fluidity of lipids on the supported region.

Finally, we examined the functionality of BLMs formed on the PFDS modified SiO_2/Si_3N_4/Si chips through the incorporation of hERG channels into the BLMs. The hERG channel is a voltage-gated potassium channel in the human heart. After incorporation of the channel, stepwise currents with a single-channel conductance of 12 pS were recorded. This conductance level was similar to reported values. The channel activities were blocked when a specific blocker was added, confirming the functional reconstitution of hERG channels into the BLMs formed in the PFDS modified Si chips.

4. Conclusions

We investigated the effects of the surface modification not only on the fluidity of lipid monolayers formed on modified septa but also on the stability of free-standing BLMs surrounded by a modified septum. The diffusion coefficient of lipid membrane on the modified surfaces was affected by hydrophobicity, lipophobicity and surface roughness. Highly amphiphobic surfaces with high water and oil contact angles lead to the formation of highly fluidic lipid monolayers on them, while a large surface roughness reduces the fluidity of the lipid monolayers. The stability of BLMs was also affected by the type of surface modification of the septa. BLMs on PFDS-modified chips had a higher mechanical stability, than BLMs that were formed on CPDS-modified chips. Since the lipid monolayers on the septa seamlessly connect with BLM in a free-standing region, the high lipid fluidity contributes to compensating potential damage to BLMs. This approach is simple and widely accessible, suggesting that it leads to an improved experimental throughput for BLM-based reconstitution systems and contributes to realization of high-throughput drug screening platforms for use in studies of ion channel proteins after a further enhancement of the bilayer stability.

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