

Simultaneous Electrophysiological and Infrared Spectroscopic Studies of Lipid Bilayer Formation

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1. Introduction

Supported bilayer lipid membranes (BLMs) have attracted attention as a model of biological membranes. The planar geometry of the BLMs allows one to investigate the structure and function of the membranes using surface sensitive techniques. However, the functionality of such membranes is restricted by their close surface proximity, which likely causes denaturation of incorporated ion-channel proteins. On the other hand, agarose gels work as a hydrophilic spacer between BLMs and solid supports [1]. They provide the planar geometry on solid supports while allowing the bilayers access to a bulk-like aqueous environment. With BLMs cushioned on agarose gels, current recordings of biological ion-channels have been successfully reported [2,3], showing the functionality of these membranes. In the present study, we have investigated simultaneous electrophysiological and infrared absorption spectroscopic (IRAS) monitoring of the self-formation process of BLMs cushioned on agarose-coated Si using IRAS in the multiple internal reflection (MIR) geometry. IRAS signals corresponding to the self-thinning of lipid solution to form BLMs was demonstrated. The functionality of the membranes was also investigated by incorporating gramicidin channels into the membranes.

2. Experimental procedures

A rectangular Si prism ($12 \times 30 \times 0.45 \text{ mm}^3$) was prepared from a double-side-polished, p-type Si(100) wafer with a resistivity of 5250 - 7050 Ωcm . The prism had 45° bevels on each of the short edges. To apply a potential to the Si prism, thin gold films were deposited onto one side of the prism (Fig. 1). Then a layer of SU8-3010 photoresist was spun onto the other side of the prism and patterned by the standard photolithography to form apertures with a diameter of 100 μm . An agarose solution was spun on the SU8 layer and then dried. The prism thus prepared was set on the bottom of a Teflon cell. BLMs were prepared in D_2O containing 0.15 M NaCl and 10 mM HEPES/NaOD ($\text{pD}=7.4$) by painting the apertures with n-decane solution containing L- α -phosphatidylcholine (PC) and cholesterol. The formation process of BLMs was monitored with membrane resistance and IR absorption spectra. The resistance was measured with the Axopatch 1D amplifier

(Axon Instruments). IR spectra were collected on a BOMEM MB-100 Fourier transform infrared (FTIR) spectrometer, equipped with a liquid-nitrogen cooled mercury-cadmium-telluride (MCT) detector. After formation of BLMs, methanol solution of gramicidin D (Sigma) was added to the cell. Current recordings of gramicidin channels were performed with the same amplifier at the sampling rate of 1 kHz.

2. Results and Discussion

Fig. 2 shows an example of IR absorption spectra after painting the apertures with an *n*-decane solution containing PC and cholesterol. A C=O stretching band of PC was observed around 1740 cm^{-1} just after the lipid application (0 min). The intensity of this band increased with time, accompanied with a peak broadening with a shoulder around 1720 cm^{-1} . After 60 min, the spectrum appeared to be a summation of component bands centered around 1720 and 1740 cm^{-1} . The band at $\sim 1720 \text{ cm}^{-1}$ was assigned to the C=O stretching mode of PC, which participated in hydrogen bonding with water, while the band at 1740 cm^{-1} was assigned to the non-hydrogen bonded C=O mode [4,5]. Since the C=O stretching bands are sensitive to changes in the polarity of local environments surrounding

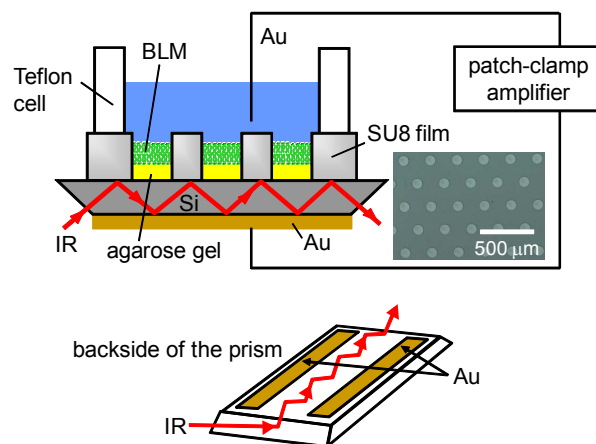


Fig. 1. A schematic of the cell used for MIR-IRAS and electrophysiological measurements. Inset shows a microscopic image of a SU-8 film prepared on a Si prism.

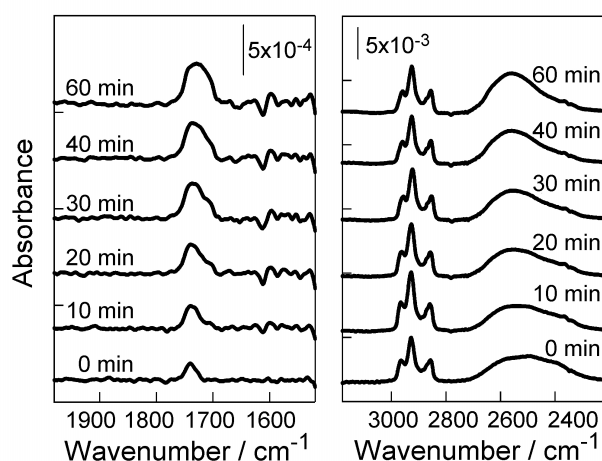


Fig. 2. IR absorption spectra during BLM formation.

phospholipids [5], such spectral changes were induced during the BLM formation.

In the higher wavenumber region, a broad band around 2560 cm^{-1} and three bands around $2860\text{--}2970\text{ cm}^{-1}$ were observed. The broad peak at $\sim 2560\text{ cm}^{-1}$, which was assigned to OD stretching mode of D_2O [6], appeared immediately after the lipid application. The band intensity increased with time, accompanied with a decrease in band width. Such narrowing may reflect the gradual changes in the ordering of water molecules surrounding the lipid phase. The three bands around $2860\text{--}2970\text{ cm}^{-1}$ were assigned to CH_x stretching modes of acyl chains [7]. Since the band intensity was about ten times higher than that of the $\text{C}=\text{O}$ modes of PC, these CH_x bands mainly arose from *n*-decane acyl chains rather than phospholipid ones. The band intensity decreased with time, suggesting that *n*-decane was slowly expelled from the surface. The simultaneously monitored membrane resistance showed a similar time course to reach a $\text{G}\Omega$ seal, suggesting that the observed time courses for the IRAS peaks reflect the self-formation process of lipid bilayer via thinning of the lipid solution.

Next, we examined the functionality of the present BLMs by incorporating gramicidin channels into the membranes. Gramicidin is a natural ion channel-forming pentadecapeptide. When gramicidin monomers form a membrane-spanning dimer, they form a channel that is permeable to monovalent cations. Addition of gramicidin channels into the aqueous solution lead to the induction of a gramicidin multi-channel current (Fig. 3). The number of open gramicidin channels was calculated to be $\sim 10^2$ based on the reported single-channel conductance ($5\text{--}6\text{ pS}$) in similar condition [17, 18]. When the aqueous phase was changed from Na^+ buffer to K^+ buffer, the membrane resistance decreased, suggesting that the BLM containing gramicidin shows higher conductance in K^+ than in Na^+ . This observation is in agreement with the ionic selectivity of gramicidin [17, 18], confirming that the present BLMs allow recording functional activities of gramicidin channels.

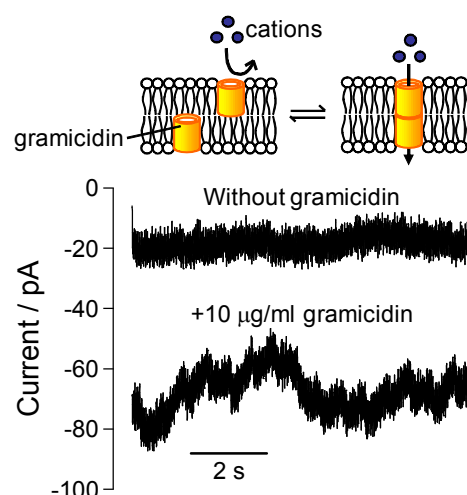


Fig. 3. An example of gramicidin channel current recorded at -70 mV from a lipid bilayer containing PC and cholesterol.

3. Conclusions

We have investigated *in situ* the formation process of lipid bilayers on agarose-coated Si surfaces by simultaneous monitoring of IRAS spectra and membrane resistance. Time-dependent changes in IRAS peaks corresponding to the self-thinning of lipid solution to form bilayer structures have been demonstrated. This method can be, in principle, applicable to various membrane systems including those containing membrane-spanning ion-channel proteins.

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