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Formation of Lipid bilayer on Ion Image Sensor and Measurement of Ion Concentration Change

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Abstract

We aimed to establish a measurement system using an artificial lipid bilayer membrane that enables multipoint measurement using a CCD ion image sensor. A supported lipid bilayer (SLB) was formed on the sensor and was observed with a fluorescence microscope. The time responses to the exchange of buffer solutions with different K^+ concentrations were measured with sensors before and after the formation of SLB. We found decrease in output potential and deceleration of the potential response after the formation of SLB in comparison with those before the formation of SLB. Addition of gramicidin made the time response after the exchange of the buffer solutions similar to that measured with the sensor before formation of SLB.

1. Introduction

Cells are the constituent units of all living organisms. Their outermost layers are cell membranes, which do not only divide cells from the external environment, but also have functions as reaction fields for transporting substances, information, and energy via membrane proteins such as ion channels. It is necessary for membrane proteins to be embedded in a lipid bilayer membrane in order to maintain their proper structures and functions. Approximately 60% of drug discovery targets are membrane proteins [1], therefore they are important research subjects in the fields of medicine and drug discovery. Ion channels are one of typical membrane proteins and occupy a major part of the drug discovery targets [ref1]. Measurement of ion current is the mainstream for the study of ion channels [2], but the improvement of throughput is demanded. In this study, we aim to establish a method using artificial lipid bilayer membranes and a semiconductor-based sensor for multipoint measurement of ion channels. We used the CCD ion image sensor with a plasticized poly(vinyl chloride) (PVC) membrane [3], and formed a supported lipid bilayer (SLB) [4], which is an artificial membrane system at solid-liquid interfaces, on the sensor. We measured the K^+ concentration and its temporal change before and after the formation of SLB on the CCD ion image sensor.

2. Experimental

2.1. Preparation of K^+ ion selective membrane and potential measurement

As the ion selective membrane on the CCD ion image sensor, we formed a PVC membrane containing valinomycin, which is an ionophore specific for K^+, following the previous study [3]. The CCD ion image sensor after formation of PVC was calibrated by using 1 mM, 10 mM, 100 mM K^+ buffer solutions. We measured time responses of the surface potential using the CCD ion image sensor, during the exchange of buffer solutions containing 1 mM and 100 mM K^+.

2.2 Formation of SLB and reconstruction of gramicidin

We used the vesicle fusion method to form SLB on the PVC membrane [5]. We prepared a vesicle suspension of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and a fluorescently labeled lipid (N-(lyssamine Rhodamine B sulfonyl)-1,2-dioleoyl-sn-3-phosphatidylehanolamine (Rb-DOPE)) (100:0.2) at the lipid concentration of 0.40 mM. We dropcast the suspension on the sensor covered with the PVC membrane, and incubated it at room temperature (~25 ºC) for 2 h. We used an epi-fluorescence microscope (epi-FM) to check the formation of SLB, and performed fluorescence recovery after photobleaching (FRAP) for the measurement of membrane fluidity. In addition, we measured time responses of the potential by using the CCD ion image sensor, during the exchange of buffer solutions containing 1 mM and 100 mM K^+. Gramicidin is a polypeptide consisting of 15 amino acid residues which show ion channel function for monovalent cations [6]. We added ethanol solution of gramicidin (1.1×10^{-12} mol/L) to SLB for reconstruction. We measured potential change after addition of gramicidin and the time response to exchange of the buffer solutions.

3. Results and discussion

Figure 1 shows epi-FM images and FRAP process of DOPC-SLB containing Rb-DOPE on the PVC membrane. Fluid and continuous SLB was formed on the PVC membrane. The output potential of the CCD image sensor decreased after the SLB formation compared with that before the SLB formation. Because SLB is assumed to be an insulating film, the potential drop also supports the formation of SLB on the sensor.

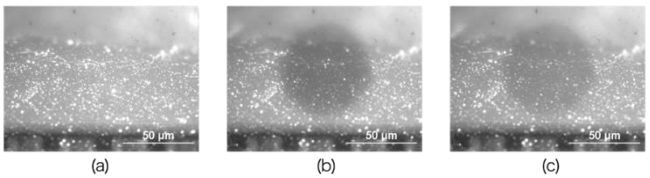

Fig. 1 Fluorescence images and time course of FRAP measurement performed on SLB on PVC membrane. (A) Before photobleaching, (b) 0 s and (c) 210 s after photobleaching.

Figure 2 shows the temporal change of the potential after the buffer exchange measured by the CCD ion image sensor before and after the formation of SLB. The potential was converted to the concentration of K^+ ($[K^+]$) on the basis of the calibration without SLB. Before the formation of SLB, the potential at $[K^+]$ = 1 mM ($-\log_{10}[K^+] = 3$) changed to that at $[K^+]$ = 100 mM ($-\log_{10}[K^+] = 1$) within 1.4 s after the exchange of the buffer solutions (Fig. 2, gray plot). After the formation of SLB, it took 35.7 s for the same potential change (Fig. 2, black plot). The potential change slowed down because the sensor surface was covered by SLB and the ions were shielded. It is known that ~1 nm thick water layer exists between the SLB and the substrate. Slow change of $[K^+]$ proceeded because of the diffusion of K^+ through the nanolayer of water.

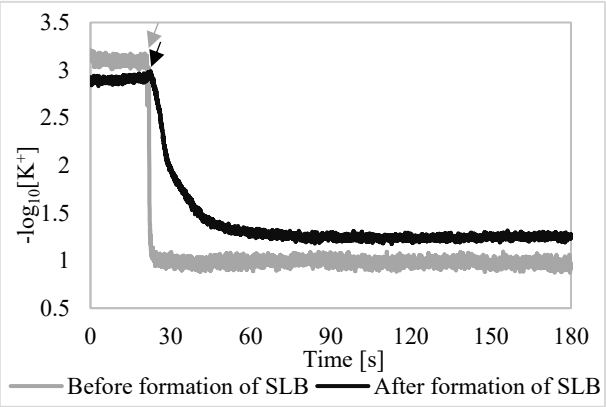

Fig. 2 The temporal change of the potential at the exchange of the buffer solutions from 1 mM to 100 mM of K^+ concentrations. Gray plot and black plots correspond to the potentials before and after formation of SLB, respectively. Black and gray arrows indicate the time at which the buffer solution was exchanged.

We added gramicidin to SLB and measured the potential change after the buffer exchange. The potential rapidly changed (Fig. 3) similar to the result without SLB (Fig. 2, gray plot). Gramicidin was reconstructed in SLB, and opened the path for K^+.

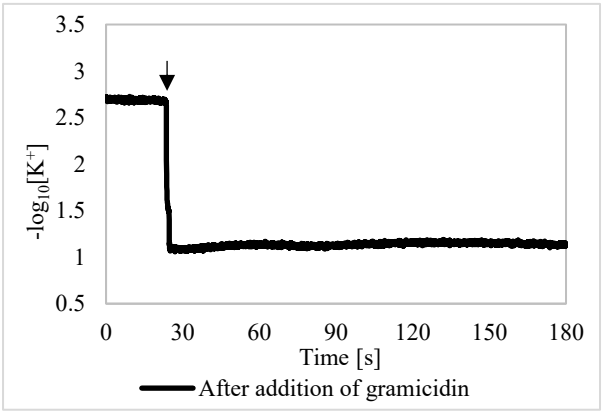

Fig. 3 The time response of potential change when exchange buffer solutions from 1 mM to 100 mM of K^+ concentrations after addition of gramicidin. Black arrow indicates the time at which the buffer solution exchange was exchanged.

4. Conclusion

We formed SLB on the ion selective PVC membrane on the CCD ion image sensor. The time responses of exchanging buffer solutions with different K^+ concentrations were measured with the sensors before and after formation of SLB. After the formation of SLB, the output potential decreased and the potential change slowed down in comparison to those before the formation of SLB. These results showed that the sensor surface was covered by SLB and the ions were shielded. After the addition of gramicidin, rapid response similar to that before the formation of SLB was observed. It is considered that ion permeation through gramicidin embedded in SLB occurred. In this study, we showed that model peptide was reconstructed in the artificial lipid membrane on the CCD ion image sensor. We expect the application to channel proteins in the future.

Acknowledgements

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