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Proton transport in supported lipid bilayer system measured with ion image sensor

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Abstract

We evaluated the H^+ transport through an artificial lipid bilayer system on a change transfer type ion image sensor. Supported lipid bilayer of dioleoylphosphatidylcholine was formed by the vesicle fusion method. We measured time course of the output voltage of the sensor during the exchange of the buffer solutions with different pH. SLB formation on the sensor slowed down the voltage response. Reconstituting gramicidin A, which is a polypeptide showing ion channel activity, recovered the voltage response. These results showed that SLB on the sensor surface efficiently shielded H^+ diffusion, and that the SLB system on the ion image sensor is available to detect H^+ channel activity.

1. Introduction

The cell membrane is the outermost layer, and divides cells from the external environment. The fundamental structure of the cell membrane is the lipid bilayer, which is a bimolecular sheet of amphiphilic lipid molecules. The hydrophobic core of the lipid bilayer effectively prevents the permeation of ions and water-soluble agents through the cell membrane. The cell membrane also functions as reaction fields for transporting substances, information, and energy in and out of cells via membrane proteins such as ion channels. Membrane proteins occupy a major part of the drug discovery targets, and thus are important research targets in the fields of medicine and drag discovery.

Artificial lipid bilayer systems, such as the free-standing bilayer lipid membrane, vesicles, and supported lipid bilayers (SLBs), have been used for the researches of cell membranes and membrane proteins [1-3]. In these experimental systems, shielding efficiency of the lipid bilayer membranes is demanded to suppress the leakage of targeted ions or water-soluble agents. For example, high-resistant “giga ohm sealing” is needed for measuring the activity of ion channels using the free-standing bilayer membrane system [1-2]. SLB is an artificial lipid bilayer formed on a solid substrate at a solid-liquid interface. SLB has a high technical affinity with solid devices and sensors. On the other hand, SLB tends to have lower sealing efficiency compared to the free-standing bilayers and vesicles [2-3].

Recently, we reported SLB formation on a charge transfer type ion image sensor [4]. SLB formed on the K^+-sensitive membrane, which is composed of polyvinyl chloride, shields K^+ sufficiently for measuring the time course after changing K^+ concentrations. In the present study, we fabricated SLB on the charge transfer type H^+ image sensor. We showed that SLB on the sensor surface effectively shielded H^+. We also investigated the effect of ion channel reconstitution into SLB on the voltage response.

2. Experimental

A charge transfer type ion image sensor with 128 x 128 pixel [5,6] was used. The sensor was treated with NaOH aqueous solution to hydrophilize the surface of the topmost Si_3N_4 layer, and then rinsed with pure water. We recorded the output voltage instead of the calibrated pH value, because the covering the sensor surface with SLB shifts the output voltage [4]. We confirmed that the sensor without SLB gave output voltages close to the Nernstian response.

Buffer solutions of pH 7.4 (100 mM KCl, 25 mM HEPES / NaOH) and pH 4.8 (100 mM NaCl, 25 mM succinic acid / NaOH) were alternately provided using a sealed flow system shown in Fig. 1. We measured time responses of the output voltage of the ion image sensor during the exchange of buffer solutions of pH 7.4 and 4.8.

We used the vesicle fusion method to form SLB on the sensor surface [3,4]. We prepared a suspension of lipid vesicles consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and a fluorescently labeled lipid (N-(lissamine rhodamine B sulfonyl)-1,2-dioleoyl-sn-3-phosphatidylehanolamine (Rb-DPPE)) (100:0.2). We dropcast the suspension on the sensor and incubated it at 45 ºC for 2 h. We checked the formation of fluid and continuous SLB by observing it with an epi-fluorescence microscope (epi-FM) and by measuring fluorescence recovery after photobleaching (FRAP) [3,4]. 2 μL of the ethanol solution of gramicidin A (GA) was added to SLB for reconstituting GA to the bilayer membrane [4].

3. Results and Discussion

Figure 2 shows the time courses of the output voltage of the sensor when the buffer solution on the sensor was changed from pH 4.8 to pH 7.4. It took ~20 s for the alternate buffer solution to reach to the sensor after the pump was started to exchange the buffer solution. The output voltage responded quickly when the alternate buffer solution reached to the sensor (black curve in Fig. 2). The half of time required for the temporal change ($t_{1/2}$) was 5.9 s. The orange curve in Fig. 2 shows the time course of the output voltage after SLB was formed on the sensor. The output voltages changed more slowly compared to those before the SLB formation; $t_{1/2}$ was 36.1 s. The result indicates that the sensor surface was covered with SLB, and diffusion of H^+ was shielded by SLB.

Figure 3 shows the effect of GA on the time courses of the output voltage after the exchange of the buffer solutions.

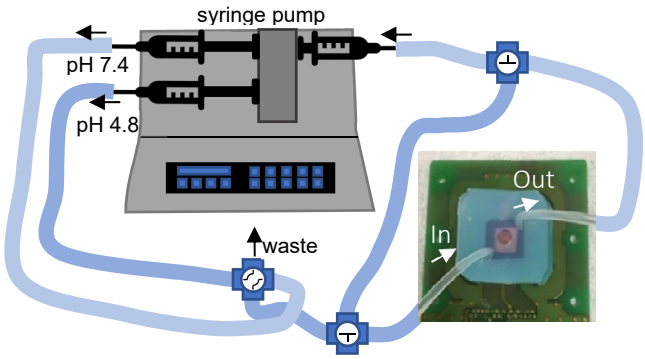

Fig. 1 Schematic illustration of the sealed flow system to exchange buffer solutions on the charge transfer type ion image sensor.

GA is a polypeptide consisting of 15 amino acid residues. Transmembrane dimer of GA shows ion channel function for monovalent cations. The sensor covered with SLB without GA had $t_{1/2}$ 13.4 s (orange curve, Fig. 3). The green and blue curves in Fig. 3 shows the voltage change 60 min after addition of GA/ethanol solution of 2.04×10^{-9} g/L and 2.04×10^{-8} g/L that corresponded to 10 and 100 GA molecules in the sensing area in each pixel of the sensor, respectively, on the assumption that all added GA was reconstituted into SLB. Adding 10 GA molecules did not much affect the voltage change; $t_{1/2}$ was 15.7 s. On the other hand, adding 100 GA molecules accelerated the voltage change, and $t_{1/2}$ became 6.0 s that was almost the same as that without SLB (Fig. 2). GA was reconstituted into the SLB, and H^+ permeated the SLB though GA.

We also found asymmetric voltage change between the buffer exchanges from pH 4.8 to 7.4 and from pH 7.4 to 4.8. Proton transport through the nanogap between the SLB and the substrate surface will be discussed.

3. Conclusions

Temporal change of the output voltage of the charge transfer type ion image sensor revealed that DOPC-SLB on the Si_3N_4 layer of the sensor shielded efficiently against H^+ permeation. We demonstrated that activity of H^+ channel though GA can be detected using the SLB on the ion image sensor.

Acknowledgements

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References

[1] M. Komiya, et al., Chem. Rec. **20**, open on web [DOI: 10.1002/tcr.201900094] (2020).

[2] A. Hirano-Iwata, et al., TrAC Trends Anal. Chem. **27**, 512 (2008).

[3] R. Tero, Materials **5**, 2658 (2012).

[4] K. Imai, et al., Jpn. J. Appl. Phys. **58**, SDDK06 (2019).

[5] M. Futagawa, et al., IEEE Trans. Electron Devices **60**, 2634 (2013).

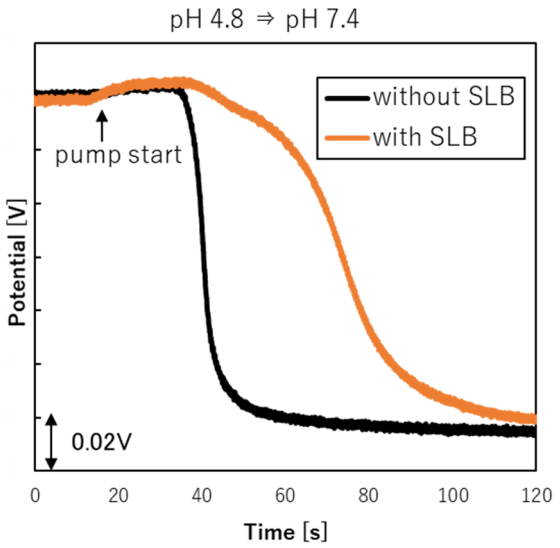

Fig. 2 Typical time course of the output voltage of the charge transfer type ion image sensor during the buffer exchange from pH 4.8 to 7.4 before (black curve) and after (orange curve) formation of DOPC-SLB.

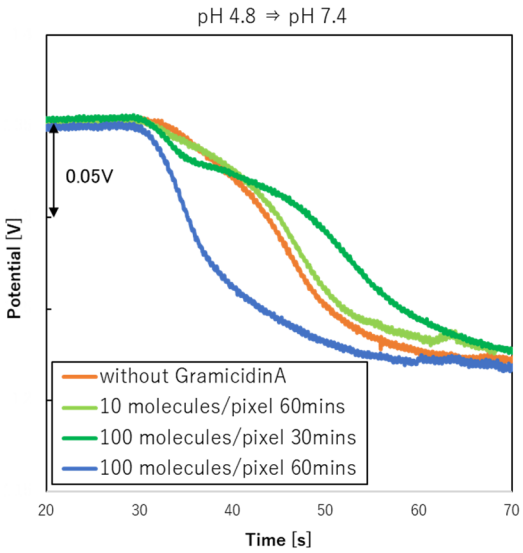

Fig. 3 Typical time course of the output voltage of the SLB-covered sensor during the buffer exchange from pH 4.8 to 7.4, before and after addition of GA.

[6] K. Sawada, et al., IEICE Trans. Fundam. Electron. Commun. Comput. Sci. **E97.A**, 726 (2014).