EXTEND THE SIZE OF BIOLOGICAL NANOPORE USING MGAININ AND PERFORIN PORES

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ABSTRACT
This paper describes two different biological nanopores reconstituted in bilayer lipid membranes (BLMs). To date, alpha-hemolysin (1.4 nm in diameter) pore has been used in the most of the nanopore sensing as the biological pores. This size of pore can detect a single-stranded DNA (ssDNA) molecule because the diameter between the pore and ssDNA is compatible. Although much larger nanopore is required for detecting proteins, secondly-structured DNA/RNA, and antigen, the variation of biological nanopores is practically nought. In this study, we show two different proteins, magainin (Mag) and human perforin (PFN), reconstituted in BLMs and measured the channel current conductances.

KEYWORDS: Nano Pore Sensing, Bilayer Lipid Membranes, Membrane Protein, Alpha-hemolysin

INTRODUCTION
Various types of membrane proteins exist in the surface of the cell membranes. A part of these membrane proteins form nano-scale pores called nano pore in the cell membranes. The pore-forming proteins can recognize and transport various substrates between inside and outside the cells. This cognitive function of nanopores is utilized in the field of biosensing, and this technique has been recently well-known as the nanopore sensing. The nanopore sensing plays an important role in the field of next-generation DNA sequencing, single molecule detection, and high sensitive biosensing. We have been developing the device to prepare BLMs by using MEMS technology and studying properties of pore-forming proteins. In the biological nanopore sensing, the pore-forming proteins are reconstituted in the BLMs and form the nanopores spontaneously as shown in Figure 1A. Then, the ions pass through the nanopore under an applied voltage, which is observed as the increase of the channel current. Besides, the translocations of target molecules via the nanopores can be detected as the blocking current.

An a-hemolysin nanopore have been studied its properties and often used for sensing of single-stranded DNA (~1.0 nm in diameter) because of the excellent size-matching of the pores (1.4 nm in diameter). Although the nanopores are expected to use very strong tools for the single molecule sensing, only a few types of the peptide have been used to date. Moreover, the available size of the nanopores is around 1 nm scale in diameter, which causes the size limitation of target molecules. Therefore, we attempt to reconstitute the different pore-forming peptides, Mag and PFN, in BLMs. The Mag is antibacterial protein existing on the skin of African clawed frog. PFN is the protein produced by killer T cell of mammals and related about the immune reaction to destroy the infected cell. We reconstituted these pore-forming proteins in BLMs and evaluated their properties.

EXPERIMENTAL
To reconstitute two different types of nanopores in BLMs, we changed the buffer contents, the protein concentration, and lipid contents. 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), 1,2-dioleoyl-sn-glycero-3-phosphatidylglycerol (DOPG) were from Avanti Polar Lipids. Mag was added at 2 μM concentrations to cis side only (the cis side was grounded and the trans side was where the potential of +100 mV was applied). The both of the sides contained the 20 μl of the buffer consisting of 1 M KCl, 10 mM Tris, pH 7 and 5.5 μl of 10 mg/ml DPhPC in decane. Then, we changed the composition of the BLMs to 5.5 μl of 10 mg/ml DOPG/DPhPC mixture (1:1, mol/mol) in decane, and conducted the same experiments to compare the manner of pore formation. PFN was added at 1 nM concentrations to cis side only, and the both sides contained the 20 μl of the buffer consisting of 0.1 M KCl, 10 mM HEPES,
pH 7 and 5.5 μl of 10 mg/ml DPhPC in decane. BLMs were prepared by using “droplets contact method” in our MEMS device as shown in Figure 1B. In this method, the two monolayers contact together and form BLM on a thin hydrophobic film (paryleneC). Ion channels or pore-forming proteins are reconstituted in the BLMs, and they allow ions to transport between chambers under the voltage gradient. The channel current signals can be measured by a voltage clamping method using by Pico Patch Clamp Amplifier (Tecella, USA). We reconstituted Mag and PFN in the BLMs and evaluated pore properties respectively.

![Figure 1: (A) An α-hemolysin reconstituted in BLM forms nanopore (1.4 nm in diameter) and ions pass through the nanopore under the potential gradient. (B) A photograph of the device fabricated by MEMS technology for preparing the BLM.](image)

RESULTS AND DISCUSSION

In the experiments of Mag, the several amplitudes of the channel current signals were observed as shown in Figure 2. The appearance of the multi-conductance suggest that Mag forms heterogeneous sized pores by origomerization with various numbers of the monomer. The conductance of the pore of Mag was about 0.1 - 4 nS in both types of BLMs. The most of the conductance values from Mag pores were nearly 1 nS. We calculated the pore size with the Hill’s equation from the conductance, which were mainly 0.5 - 3 nm in diameter. The time of pore formation depended to the lipid contents. Mag formed pores in shorter time in DOPG/DPhPC mixture membrane than only the DPhPC membrane. Moreover, Mag formed pores with lower concentration in mixture membrane than only DPhPC. This result implies that the positive charge of Mag molecule at four lysine residue interacted with negative charge of DOPG molecules. In contrast, Mag should not have the interaction with neutral DPhPC and the pore formation need much longer time.

Similarly, PFN formed heterogeneous sized pores of which conductances were about 0.1 - 4 nS, and the most of the conductances were nearly 1 nS. The pore diameter calculated by the Hill’s equation was 5 - 10 nm, and most of these pores were nearly 5 nm. The difference in Hill’s diameter compared to Mag pores is caused by the difference of electrolyte concentration. Moreover, we observed that PFN formed the pores only in the presence of Ca^{2+} ion, which is already known. It is thought that the Ca^{2+} ion change the structure of PFN monomer, and this monomer come to be able to bind with lipid membrane. As described above, we succeeded to reconstitute the nanopores of which diameter were about 0.5 - 3 nm and 5 - 10 nm by Mag and PFN.
CONCLUSION
We reconstituted two different types of nanopores in BLMs and evaluated the properties of these pore-forming proteins. Both Mag and PFN formed heterogeneous sized pores, which caused the increase of the channel current. These larger pore size than α-hemolysin pores are believed to be used for the detection of the larger molecule than about 1 nm in diameter. Therefore, we will detect a wider range of molecules by using these two different types of nanopores.

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REFERENCES

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