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Reverse osmosis membranes based on a supported lipid bilayer with gramicidin A water channels

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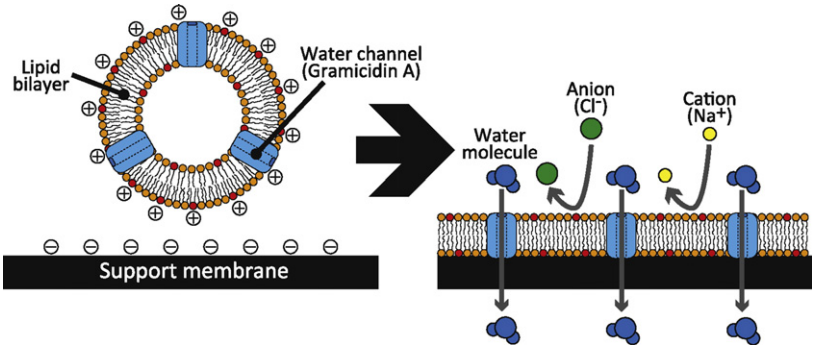
HIGHLIGHTS

- A supported lipid bilayer (SLB) was used as a biomimetic reverse osmosis membrane.
- Gramicidin A was used as a water channel molecule.
- The formed SLB showed high hydraulic pressure resistance and salt rejection.
- Gramicidin A effectively improved the water permeability of the SLB.

GRAPHICAL ABSTRACT



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ABSTRACT

This study presents the fabrication of a biomimetic reverse osmosis (RO) membrane from a supported lipid bilayer (SLB) and gramicidin A (GA) onto polymeric substrates via electrostatic interaction and by applying hydraulic pressure. GA, a cation-selective ionophore, has high permeability for water molecules and monovalent cations in a lipid bilayer, and was therefore used as a water channel molecule. Cationic liposomes containing GA were electrostatically adsorbed onto an anionic polymeric nanofiltration membrane. The adsorbed liposomes were ruptured by applying hydraulic pressure and transformed into flat lipid bilayers. SLB formation was confirmed by salt rejection measurement and fluorescence recovery after photobleaching. The defect-free SLB was formed on the polymeric nanofiltration membrane by applying over 0.15 MPa pressure. Phosphorus quantification showed that the formed SLB was not composed of a single lipid bilayer but approximately six lipid bilayers. GA incorporation into the SLB and control of the GA conformation on the SLB effectively improved water permeability. Moreover, the GA-incorporated SLB showed high NaCl rejection (>97%). Monovalent cations, Na^+ ions, were rejected to maintain electric neutrality. The formed SLB showed hydraulic pressure resistance and high salt rejection at adequate levels for RO membranes.

Abbreviations: RO, reverse osmosis; GA, gramicidin A; SLB, supported lipid bilayer; FRAP, fluorescence recovery after photobleaching; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMTAP, 1,2-dimyristoyl-3-trimethylammonium propane; TFE, 2,2,2-trifluoroethanol; CD, circular dichroism; CLSM, confocal laser scanning microscope; SD, standard deviation.

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1. Introduction

Desalination processes using reverse osmosis (RO) membranes are widely used because of their space- and energy-saving performance. However, improvement in the performance of commercially available RO membranes, such as polyamide composite membranes and cellulose triacetate membranes, has been limited [1].

Biomimetic approaches have attracted attention for the development of advanced materials. Biological membranes provide sophisticated functions, such as molecular recognition and selective permeation, achieved mainly by membrane proteins in a lipid bilayer [2]. Aquaporins are water channel proteins in a lipid bilayer that are selectively permeable to water molecules with 0.3 nm pores and binding sites for water molecules [3]. The water permeability of an aquaporin-incorporated lipid bilayer is theoretically 100 times higher than that of a polyamide RO membrane (assuming 5 mol% aquaporin in the lipids) [4,5]. Although aquaporins have very high permeability, their complex three-dimensional structures and the fragility of the proteins restrict their industrial applications. One analogous biological water channel is gramicidin A (GA), which is a cation-selective ionophore derived from *Bacillus brevis* that is permeable to water molecules and monovalent cations [6,7]. GA forms a cylindrical dimer with a 1.5 nm diameter and 0.4 nm pore in a lipid bilayer [8], and disturbs the ionic balance across cell membranes, resulting in cell death. The water permeability of GA is comparable to those of aquaporins [5,9]. Because GA is a peptide, its molecular structure is much simpler than the molecular structures of aquaporins, and reconstitution and incorporation into lipid bilayers are simple.

Because the structures of biological membranes are complex and fragile, supported lipid bilayers (SLBs) have been developed [10–13]. The advantages of biomimetic membranes are their ability to control the membrane composition, their simple structure, and their easy preparation. SLBs are physically stable and can be analyzed by physical, electrostatic, and optical methods. Therefore, SLBs have been studied as platforms for analysis of biological membranes and biosensors [14–16].

In the last decade, several fabrication techniques for biomimetic membranes using aquaporins as water channels for water purification have been reported [17–29]. Kumar et al. fabricated an aquaporin-incorporated polymersome using an amphiphilic block copolymer [18]. Other groups have developed SLBs as water purification membranes using the same block copolymer [22,23]. These membranes are fragile at high hydraulic pressures in practical uses because the membranes were fabricated in large pores, relative to the thickness of the lipid bilayers, of ultrafiltration membranes. Recently, direct immobilization of aquaporin-incorporated liposomes [24,26–28] and polymersomes [25] on support membranes have been reported. Although they improved mechanical strength, these methods could not achieve the high water permeability of the aquaporins. Kaufman et al. and Li et al. developed SLBs using lipid bilayers and aquaporins on a polymeric nanofiltration membrane via electrostatic interactions [19,21,29]. They achieved sufficient hydraulic pressure resistance to prevent lipid bilayer collapse during practical RO processes; however, they did not achieve high water permeability, salt rejection, and specific effectivity of the water channel.

In this study, we fabricated a biomimetic RO membrane using an SLB and GA. GA is generally permeable to monovalent cations, such as Na^+ or K^+ contained in sea water, and not permeable to anions (e.g., Cl^- and SO_4^{2-}) and divalent cations (e.g., Mg^{2+} and Ca^{2+}) [7]. In the RO process, it is unlikely that only cations permeate through an RO membrane because it is necessary to maintain electric neutrality. Therefore, it is expected that a GA-incorporated SLB rejects all ions, including monovalent cations. Cationic liposomes containing GA were electrostatically adsorbed onto an anionic polymeric nanofiltration membrane. They were then ruptured by applying hydraulic pressure, and transformed into a flat lipid bilayer, an SLB (Fig. 1). The lipid fluidity on the formed SLB was evaluated using a fluorescence recovery after photobleaching (FRAP) assay. The water flux and salt rejection of the formed SLB were measured by applying a typical hydraulic pressure for the RO process [30].

2. Experimental

2.1. Preparation of liposomes

Unless specified otherwise, all chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used as received. Aqueous solutions were prepared with Milli-Q water. Cationic liposomes were prepared via the extrusion of frozen and thawed liposomes [31]. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC; NOF, Tokyo, Japan) and 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP; Avanti Polar Lipids, Alabaster, AL, USA) were used as neutral and cationic lipids, respectively. DMPC and DMTAP were dissolved in chloroform in an 8:2 molar ratio. GA (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in methanol or 2,2,2-trifluoroethanol (TFE) to control the molecular conformation in the lipid bilayers. The lipid solution and GA solution were mixed at an equal volume ratio, and dried into a lipid film by removing the solvent under vacuum. The final GA concentration in the liposomes was 5 mol%. Liposomes were formed by dispersing the film in Milli-Q water at 45 °C above the phase transition temperature of DMPC. The liposome suspension was frozen in liquid nitrogen and thawed in water five times. The resulting liposomes were then extruded through 0.1-μm-pore double-stacked polycarbonate track-etched membranes (Nuclepore, GE Healthcare UK, Little Chalfont, UK) 10 times at 45 °C. After extrusion, the pH of the liposome suspension was adjusted to 2.0 using aqueous HCl. Under low pH conditions, both the electrostatic interaction between lipids and the support membrane and the electrostatic repulsion among liposomes or lipid bilayers increase [19] because the diester phosphate groups (pKa = ~2) of DMPC molecules are protonated and the DMPC molecules have an overall positive charge. When TFE was used as the solvent for GA, the liposome suspension was heated at 65 °C overnight to induce the reconstitution of

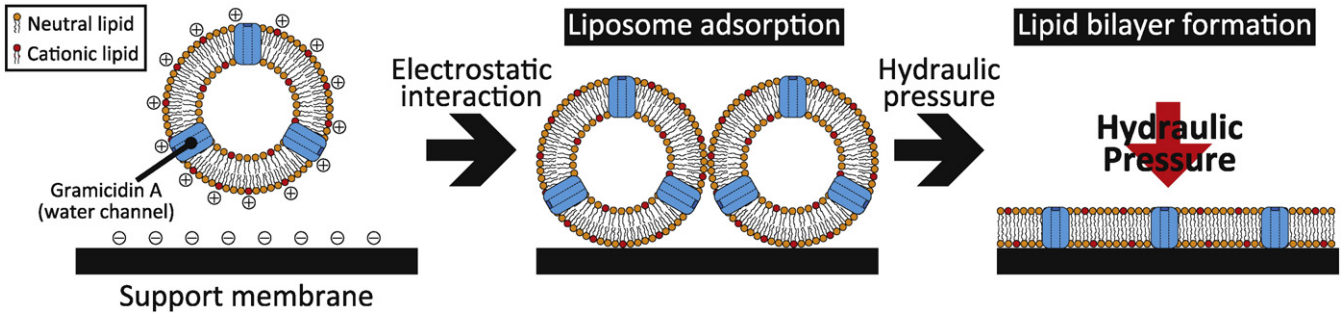

Fig. 1. Schematic diagram of SLB formation via electrostatic interactions and by applying hydraulic pressure.