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Osmotic Pressure Control in Response to a Specific Ion Signal at Physiological Temperature Using a Molecular Recognition Ion Gating Membrane

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Osmotic pressure is very important, both in artificial membranes and in biomembranes. The control of the osmotic pressure of blood relates to sympathetic nerve activity.[1] Recently, it has been reported that osmotic pressure drives phage DNA to host cells.[2] The balance of osmotic pressure and surface tension determines the volume, surface area, and form of liposomes.[3] In this way, osmotic pressure plays many important roles and is controlled by molecular signals in biosystems. Likewise, osmotic pressure is important in engineering. It is the main driving force, along with hydrostatic pressure, in the transport equation for membranes based on nonequilibrium thermodynamics,[4] and it drives the volume flux of solvents. This solvent flux has been applied to drug delivery systems, known as an "osmotic pump".[5] Therefore, if an artificial membrane can control the osmotic pressure in response to a specific molecular signal at physiological temperature, this membrane not only will be expected to have many applications, such as drug delivery, gene delivery, and microactuator, but also will be of interest because of its similarity to biomembranes. Some artificial gating membranes have been developed to mimic functions of biomembranes. These membranes can change hydrostatic-pressure-driven flux or solute diffusivity in response to stimuli such as temperature, pH, substance concentration, and light.[6] However, few gating membranes[7] exist to control the osmotic pressure.

We have developed a molecular recognition ion gating membrane that can control its pore size in response to a specific ion signal. The copolymer of *N*-isopropylacrylamide (NIPAM) and benzo[18]-crown-6-acrylamide (BCAm) was grafted onto the pore surface of this membrane. NIPAM is a thermosensitive polymer and acts as an actuator by its volume phase transition. BCAm has a crown ether receptor and works as an ion sensor. Thus, the membrane captures only the specific ion whose size fits the cavity of the crown ether receptor and swells its grafted copolymer. We have reported that the gating membrane controlled the hydrostatic-pressure-driven flux[8] or diffusivity of solute molecules[9] in response to the specific ion signal at a constant temperature. Here, we show the osmotic pressure control function of the molecular recognition ion gating membrane.

The concept of the present study is shown in Figure 1a. When Ba^{2+} is present, the crown ether receptors of the grafted copolymer capture Ba^{2+}, the grafted copolymer swells, and then the pores close; hence, osmotic pressure is generated. When Ca^{2+} is present, the crown ether receptor does not recognize Ca^{2+}; thus, the grafted copolymer shrinks, and the pores open. As a result, osmotic pressure disappears. As shown in Figure 1b, osmotic pressure is also generated by a concentration gradient of other solutes, such as dextran, in addition to the signal ion when the pores of the membrane are closed in response to the ion signal.

The membrane was prepared by the peroxide plasma graft polymerization method, as described in an earlier publication.[8,10]

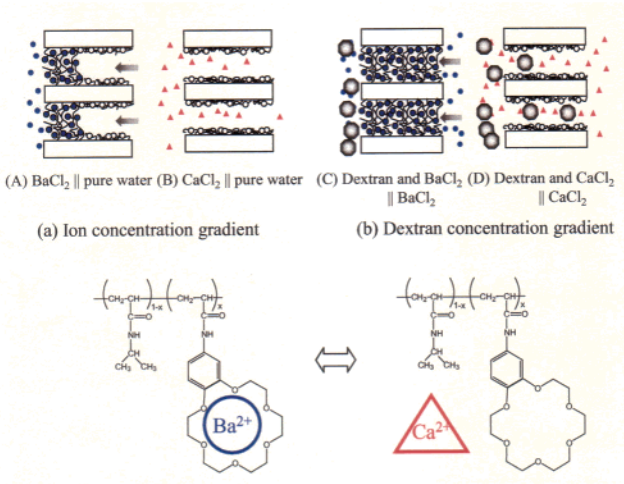

Figure 1. Concept of the osmotic pressure control by the molecular recognition ion gating membrane. The components in the solution chamber and solute chamber are expressed as "solution chamber || solvent chamber". (a) Ion concentration gradient. (b) Dextran concentration gradient.

Porous high-density polyethylene film (pore size, 0.2 μm; thickness, 100 μm) was used as a substrate, and plasma treatment power and time were 30 W and 1 min, respectively. Various graft polymerizations onto the pore surface were attempted.[11] Among these polymerizations, plasma graft polymerization can make homogeneous grafting onto pores.[12] Hence, the prepared membrane, of which the grafted amount was 1.01 mg/cm^2, had suitable morphology to generate osmotic pressure. Osmotic pressure was measured using the osmometer cell[13] at 38, 39, or 43.5 °C. The osmometer had a solution chamber and a solvent chamber, the membrane being placed between these two chambers. The solution chamber was filled with an ion solution or an aqueous mixture of ion and polymer solution and was closed by a valve to prevent the inflow of solvent. The increase in pressure inside the solution chamber was detected by a pressure sensor (KEYENCE, AP-12A) and recorded by a personal computer at intervals of 1 min. The solvent chamber was filled with pure water or ion solution. Dextran T500 (M_w = 470 000) was used as the solute to generate the osmotic pressure, and $BaCl_2$ and $CalCl_2$ were used as signal ions.

Figure 2a shows the osmotic pressure change with time at 38 °C. When the solution was aqueous $BaCl_2$ and the solvent was pure water, the osmotic pressure increased to 80 kPa. Subsequently, it decreased gradually because of ion diffusion to the solvent chamber. The decrease in osmotic pressure is a well-known phenomenon that occurs in a membrane whose reflection coefficient is less than 1.[4] In contrast, when the solution was aqueous $CaCl_2$, no osmotic pressure was generated. From this, it is concluded that the membrane recognized Ba^{2+} and generated osmotic pressure autonomously.

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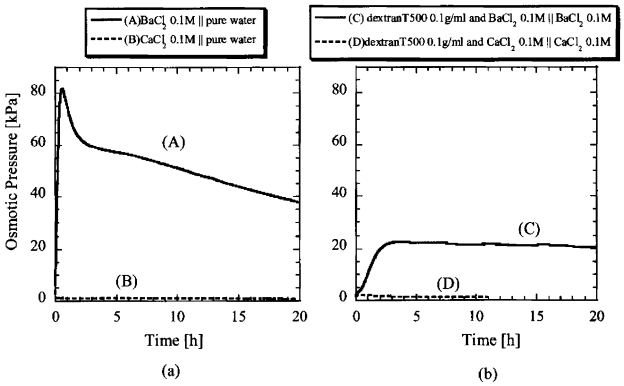

Figure 2. Osmotic pressure change with time at 38 °C. (a) Ion concentration gradient. (b) Dextran concentration gradient.

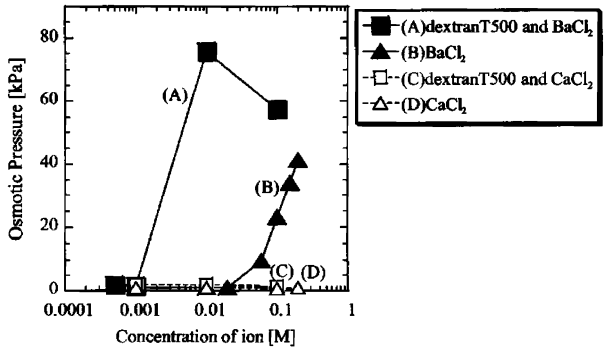

Figure 3. Relationship between osmotic pressure and ion concentration. Osmotic pressure was defined as the osmotic pressure 15 h after starting the measurement. The concentration of dextran T500 and the temperature were kept at 200 000 ppm and 43.5 °C, respectively. The components in the solution chamber and solvent chamber were as follows: (A) dextran T500 200 000 ppm and $BaCl_2$ || $BaCl_2$, (B) $BaCl_2$ || pure water, (C) dextran T500 200 000 ppm and $CaCl_2$ || $CaCl_2$, and (D) $CaCl_2$ || pure water.

In addition, when the solution was an aqueous mixture of 1×10^5 ppm dextran and 0.1 M $BaCl_2$, and the solvent was an aqueous 0.1 M $BaCl_2$, osmotic pressure was generated by the dextran concentration gradient, as shown in Figure 2b. At this time, the diffusivity of dextran was much smaller than that of the ion; thus, osmotic pressure remained constant at 20 kPa for a long period. As compared with the solution containing Ba^{2+}, the osmotic pressure disappeared when $CaCl_2$ was used instead of $BaCl_2$. Therefore, the osmotic pressure can be controlled by a second solute in addition to the signal ion.

To investigate the critical concentration at which the membrane begins to generate osmotic pressure in response to the ion, the relationship between osmotic pressure and ion concentration at 43.5 °C was investigated, as shown in Figure 3. Osmotic pressure was defined as the osmotic pressure after 15 h from the start of the measurement, because, as shown in Figure 2, the osmotic pressure changed at a constant rate. The concentration of the dextran was 2×10^5 ppm. When the driving force was either the ion concentration gradient or the dextran concentration gradient, the solution containing $BaCl_2$ generated osmotic pressure, whereas the solution containing $CaCl_2$ did not generate osmotic pressure at any concentration. The difference between the ion solution containing dextran and the ion solution was the critical concentration at which osmotic pressure began to be generated. The solution containing dextran generated osmotic pressure at a lower concentration of $BaCl_2$ than the solution containing only $BaCl_2$. This is because dextran was so large that it was fully rejected by the membrane

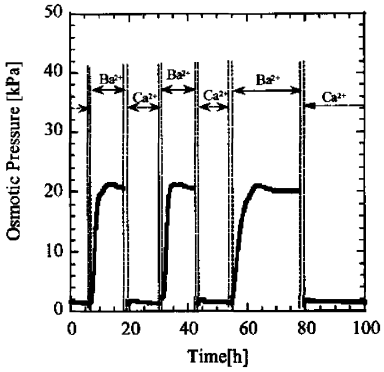

Figure 4. Osmotic pressure change with ion change. The components in the solution and solvent chamber were dextran T500 and $BaCl_2$ || $BaCl_2$ or dextran T500 and $CaCl_2$ || $CaCl_2$, alternately. The concentration of dextran T500 and the temperature were 100 000 ppm and 39.0 °C, respectively.

when the pores of the membrane became slightly smaller, whereas the size of the ion was so small that the ion was rejected only when the pores closed perfectly. Using a second solute such as dextran in addition to the signal ion, we can control the critical concentration at which the membrane begins to generate osmotic pressure.

Repeatability of response function is also important. Figure 4 shows the osmotic pressure change with time in response to alternate Ba^{2+} and Ca^{2+} signals. The interval to switch the solution was 1 or 1.5 h; the dextran concentration was 1×10^5 ppm and ion concentration was 0.1 M both of $BaCl_2$ and of $CaCl_2$, and the temperature was kept at 39 °C. The osmotic pressure of the solution containing $BaCl_2$ repeatedly generated the value of 20 kPa, whereas no osmotic pressure of the solution containing $CaCl_2$ was generated. This response occurred reproducibly.

In summary, a molecular recognition gating ion membrane captured Ba^{2+} and generated osmotic pressure in response to Ba^{2+} autonomously and reversibly. The concentration gradient of both the ion and the other solute such as dextran could be used as the driving force; using a dextran concentration gradient, we can control the critical concentration and the duration time of the osmosis response.

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