

Fig. 2. Photograph of 0.1 wt% poly-NIPAM-co-BCAm showing that the solutions of varying ethanol mole ratio were different without any ion ((A)–(D)) and with 10 mM BaCl₂ ((E)–(G)). Ethanol concentrations were (A), (E) pure water, 0 mol%, (B), (F) 9.3 mol%, (C), (G) 23.5 mol%, (D) 100 mol%. Only (B) became cloudy.

and (D) contained no ions. The aqueous solution in (A) was homogeneous, but at 9.3 mol% in (B) became cloudy. With increasing ethanol mole ratio, 23.5 and 100 mol% in (C) and (D), respectively, the solution became homogeneous again. This is a typical reentrant phase transition behavior in which the polymer starts swelling, then shrinks with changing solvent composition, and finally swells again at a constant temperature. On the other hand, the solutions in (E), (F) and (G) contained 10 mM BaCl₂. Because the solubility of BaCl₂ decreased with increasing ethanol mole ratio, the equivalent amount of 10 mM BaCl₂ did not dissolve in pure ethanol. At any

concentration of ethanol, the solutions of (E), (F) and (G) remained dissolved, which means that reentrant phase transition behavior disappeared because of ion addition.

The phase transition of poly-NIPAM-co-BCAm was explored in more detail by turbidity measurements. The absorbance of poly-NIPAM-co-BCAm solutions in which the ethanol concentration was different and contained 10 mM BaCl₂ was measured as a function of temperature, as shown in Fig. 3(A) and (B). At low ethanol concentration, phase separation occurred sharply at the LCST, as shown in Fig. 3(A), while it changed to continuous phase separation at

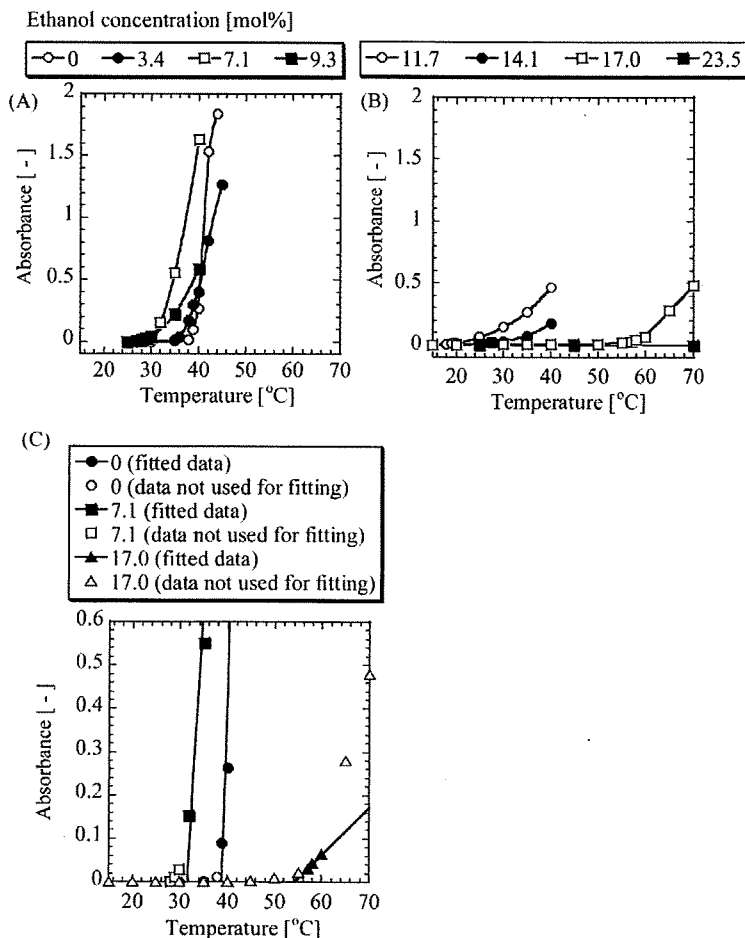


Fig. 3. Temperature dependence of the turbidity of poly-NIPAM-co-BCAm in various concentrations of aqueous ethanol solution that contained 10 mM BaCl₂. (A) Ethanol concentration was varied between 0 and 9.3 mol%. (B) Ethanol concentration was varied between 11.7 and 23.5 mol%. (C) LCST was defined as the x-intercept of the tangential line of each turbidity curve.

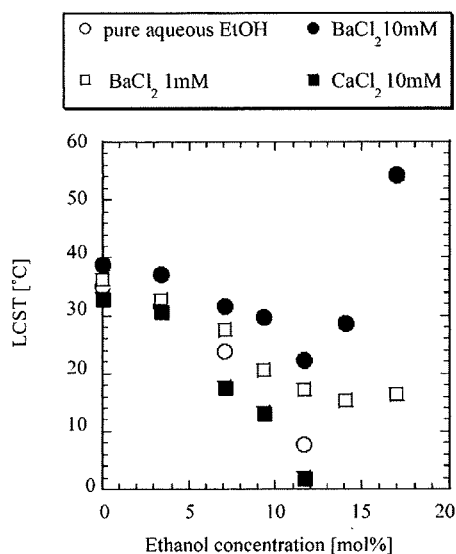


Fig. 4. Temperature–ethanol concentration phase diagram of poly-NIPAM-co-BCAm in response to signal ions. Ethanol mole ratio was varied between 0 and 20 mol%. The concentration of BaCl₂ was 1 and 10 mM. As a control experiment, pure solvent and 10 mM CaCl₂ were used.

high ethanol concentration, as shown in Fig. 3(B). Thus, the LCST was defined as the intercept temperature of the tangential line of each turbidity curve, as shown in Fig. 3(C). In addition, the LCSTs shifted to lower temperature with increasing ethanol concentration, as shown in Fig. 3(A), and then shifted oppositely to higher temperature above 11.7 mol% of ethanol, as shown in Fig. 3(B).

By determining LCSTs of poly-NIPAM-co-BCAm solution in which the analyte ion concentration is varied, the change of phase diagram in response to signal ions was obtained, as shown in Fig. 4. In the case of pure solvent or 10 mM CaCl₂ solution, the phase transition behavior of poly-NIPAM-co-BCAm was almost the same as that of poly-NIPAM already reported previously [19,20]. When BaCl₂ was added instead of CaCl₂, the LCST shifted higher, the shift width becoming higher with increasing ethanol concentration in the region where ethanol concentration was below ca. 12 mol%. This is probably because the complex formation constant of BCAM increased with increasing ethanol concentration [30].

3.2. Change of phase diagram and reentrant phase transition of poly-NIPAM-co-BCAm by a specific ion signal

It is reported that poly-NIPAM has an LCST at low concentrations of ethanol and a UCST at relatively high concentrations of ethanol in aqueous ethanol solutions. Between the LCST (LCST₁) and UCST lines, there is a globule-state region where poly-NIPAM is always insoluble at any temperature. Interestingly, the phase diagram of poly-NIPAM-co-BCAm in aqueous ethanol solution was different from that of poly-NIPAM in aqueous ethanol [20] even without adding ions. It has LCST₁, which we focused on in Fig. 4 and a second LCST (LCST₂) replaced with UCST, but there is a coil-state region of an insoluble state at any temperature.

When 10 mM CaCl₂ solution was used, the phase diagram was almost the same as that of pure solvent. On the other hand, in the case of BaCl₂, the globule-state region became smaller with increasing BaCl₂ concentration, and then two coil-state regions finally connected and became one region. Thus, the LCST lines became concave. As a result, the reentrant phase transition behavior of poly-NIPAM at around room temperature was lost because of the Ba²⁺ signal in the case of poly-NIPAM-co-BCAm.

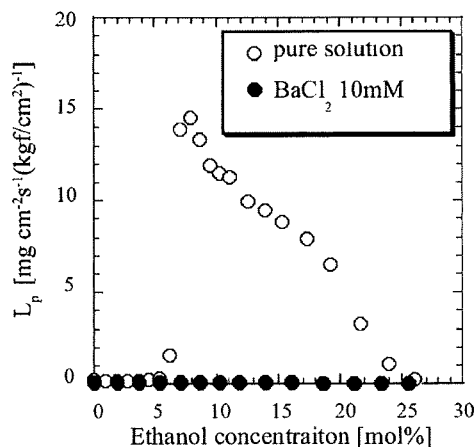


Fig. 5. Dependence on ethanol concentration of the permeability coefficient, L_p , of aqueous ethanol solution containing no ion or 10 mM BaCl₂ through a molecular recognition ion gating membrane at 24 °C.

A mechanism for the reentrant phase transition of poly-NIPAM [16,18] and volume phase transition of poly-NIPAM gel [17,19] has been suggested in some previous research, but it is not clear even now. We speculate that formation of a complex between the crown ether ring of BCAM and Ba²⁺ decreased the cooperativity of the phase transition along the NIPAM chain. Therefore, the size of the globule-state region became smaller and the two coil-state regions connected to each other.

3.3. Reentrant phase transition behavior of the pressure-driven flux through the molecular recognition ion gating membrane

Fig. 5 shows the pressure-driven flux of pure water and 10 mM BaCl₂ at 24 °C when the ethanol concentration was varied between 0 and 25 mol%. In the case of pure water, the pores of the membrane opened at around 7 mol% of ethanol, and then the flux gradually decreased. The pores finally closed at 25 mol% of ethanol. On the other hand, the pores of the membrane always closed at any concentration of ethanol at 24 °C in the presence of 10 mM BaCl₂. That is to say, the reentrant phase transition was lost because of 10 mM BaCl₂. This behavior agrees well with that of poly-NIPAM-co-BCAm as shown in Fig. 4, because the behavior of the membrane is basically determined by the properties of grafted poly-NIPAM-co-BCAm. At 12 mol% of ethanol in Fig. 4, the LCST was below 24 °C, but the phase transition gradually occurred, as shown in Fig. 3, and the copolymer shrank a little; thus, the pores were closed even at this ethanol mole ratio.

3.4. LCST/LCST-type reentrant phase transition of the pressure-driven flux through the gating membrane without the ion signal

The operating temperature was also varied between 13 and 50 °C, as shown in Fig. 6. The LCST shifted to lower temperature with increasing ethanol mole ratio. Note that the LCSTs were defined at the onset temperature of the permeation flux in the permeation experiments of this study. At the same time, the maximum pressure-driven flux through the membrane decreased and finally became zero, where the pores always closed at any concentration of ethanol because of the swelling of the grafted poly-NIPAM-co-BCAm, a behavior that coincided with the reentrant phase transition of poly-NIPAM-co-BCAm.

One unique point of poly-NIPAM-co-BCAm was also clarified from Fig. 6 during the reentrant phase transition of poly-NIPAM-co-BCAm. As previously reported [20], poly-NIPAM showed an

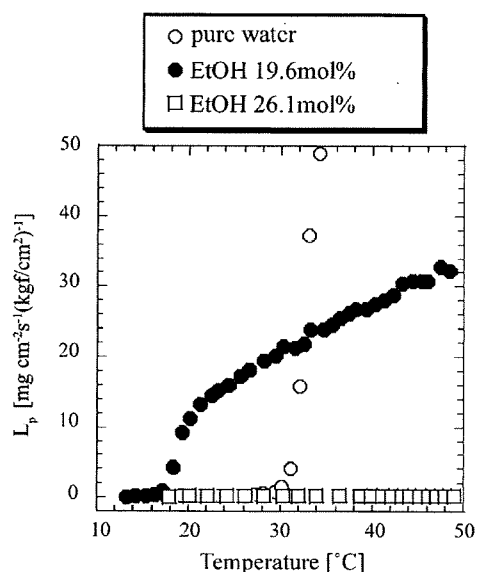


Fig. 6. Temperature dependence of the permeability coefficient of aqueous ethanol solutions through a molecular recognition ion gating membrane. Ethanol concentrations were 0, 19.6 and 26.1 mol%.

LCST/UCST-type reentrant phase transition in the mixed solvent of ethanol and water. On the other hand, poly-NIPAM-co-BCAm showed an LCST₁/LCST₂-type reentrant phase transition, because the membrane showed an LCST at 17 °C at 19.6 mol% of ethanol (Fig. 6). Introduction of BCAM apparently affected the globule-to-coil transition of poly-NIPAM at the UCST. In the same previous research, methanol and acetone showed only an LCST₁/LCST₂-type reentrant phase transition, while ethanol, 1-propanol, 2-propanol, DMF and DMSO showed LCST/UCST-type reentrant phase transitions. In the LCST/UCST systems, the hydrophobic interaction was focused at the UCST because alcohols that have a UCST are more hydrophobic than methanol [20], and it is speculated that alcohols interact with poly-NIPAM by hydrophobic interactions. We speculate that the introduction of the hydrophilic crown ether ring of BCAM may contribute to weakening this hydrophobic interaction, and ethanol may behave like methanol in the case of poly-NIPAM-co-BCAM.

3.5. The effect of ion species and concentration on LCST shifts of the gating membrane

Crown ether receptors contained in the membrane recognize different ion species, which leads to a membrane response to a specific ion signal. Fig. 7 shows the temperature dependence of the pressure-driven flux in the presence of various ions such as Ba²⁺, Sr²⁺, Ca²⁺ and K⁺ in the mixed solvent of water and ethanol in which the ethanol concentration was 19.6 mol%. The order of the width of LCST shift was Ba²⁺ > Sr²⁺ = K⁺ > Ca²⁺ = no ion. Compared with our previous results in aqueous solution, some points were different. First, the shift width of the LCST in the case of Sr²⁺ was larger than that in the case of K⁺ in aqueous solution [1], while they showed almost the same value in the mixed solvent of water and ethanol. Second, 100 mM aqueous solutions were used in the previous study, while 10 mM solutions were used in the mixed solvent of water and ethanol. However, the LCSTs shifted almost by the same amount for each ion.

Fig. 8 shows the temperature dependence of the pressure-driven flux at different concentrations of BaCl₂ in the mixed solvent of water and ethanol, in which the ethanol concentration was 19.6 mol%. The LCST shifted to higher temperature with increas-

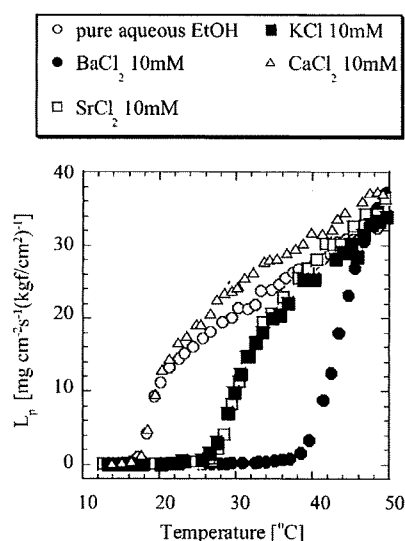


Fig. 7. Temperature dependence of the permeability coefficient of aqueous ethanol solutions containing different ion species through a molecular recognition ion gating membrane. Concentrations of ethanol and ion were 19.6 mol% and 10 mM in all cases, respectively.

ing concentration of BaCl₂. On the other hand, the pores always closed because of the swelling of the grafted copolymer when the ethanol concentration was 26.1 mol%, as shown in Fig. 9. Captured ions by the crown ether receptors do not affect the flux.

3.6. Sensitivity enhancement of the gating membrane in an aqueous ethanol solution

The LCST shift width in the presence of recognition ions was different between water and aqueous ethanol solutions, as shown in Fig. 10. In the case of an aqueous solution, the LCST shift between pure water and 100 mM BaCl₂ was almost 20 °C, while the LCST shift between pure solvent and 30 mM BaCl₂ was greater than 30 °C in the case of an aqueous ethanol solution of 19.6 mol%.

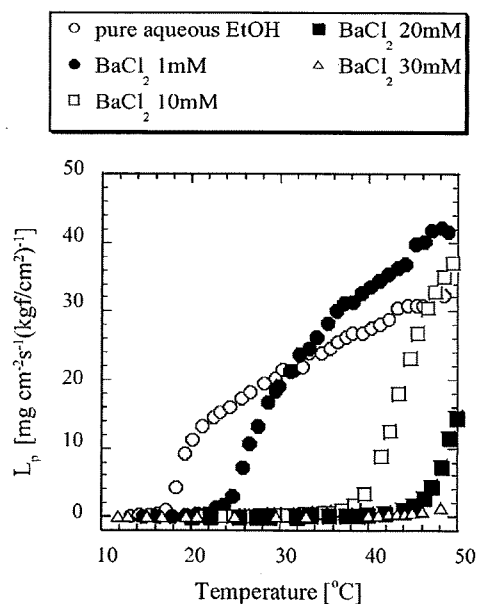


Fig. 8. Temperature dependence of the permeability coefficient of aqueous ethanol solutions containing BaCl₂ of different concentrations through a molecular recognition ion gating membrane. Concentration of ethanol was 19.6 mol% in all cases.

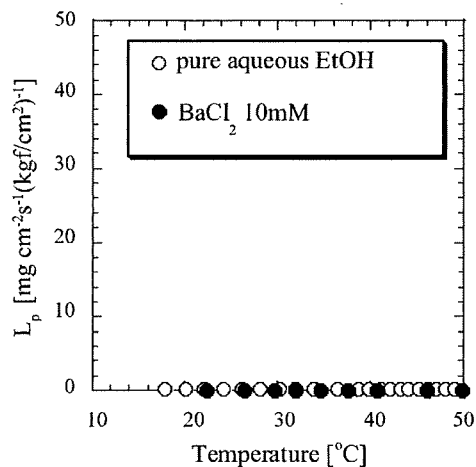


Fig. 9. Temperature dependence of the permeability coefficient of aqueous ethanol solutions containing 10 mM BaCl_2 or no ion. Concentration of ethanol was 26.1 mol% in both cases.

To compare the sensitivity of the membrane to BaCl_2 in a more direct way, the dependence of the permeation flux on BaCl_2 concentration was investigated in aqueous 19.6 mol% ethanol solution and pure water, together with that on CaCl_2 concentration at a temperature that was 10 °C higher than each LCST (Fig. 11). In the case of the ethanol solution, the permeation flux decreased from 1 mM BaCl_2 to 10 mM BaCl_2 , while it decreased from 10 mM BaCl_2 to 100 mM BaCl_2 . Thus, the sensitivity of the membrane to BaCl_2 in the aqueous ethanol solution is roughly 10 times higher than that in the aqueous solution. The concentration of CaCl_2 did not change the permeation flux, which shows that this phenomenon occurred as a result of molecular recognition by the crown ether receptors. This high sensitivity and stability of the membrane and its response in solutions containing organic solvents are unique and expected to

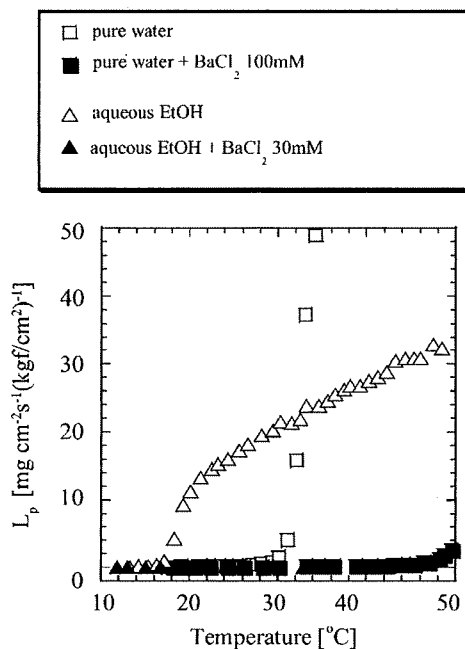


Fig. 10. Temperature dependence of the permeability coefficient of water (0 mol%) or aqueous ethanol solution (19.6 mol%) containing BaCl_2 through a molecular recognition ion gating membrane. Except for the plots of aqueous 100 mM BaCl_2 , all of the data have already been shown in Figs. 4 and 6.

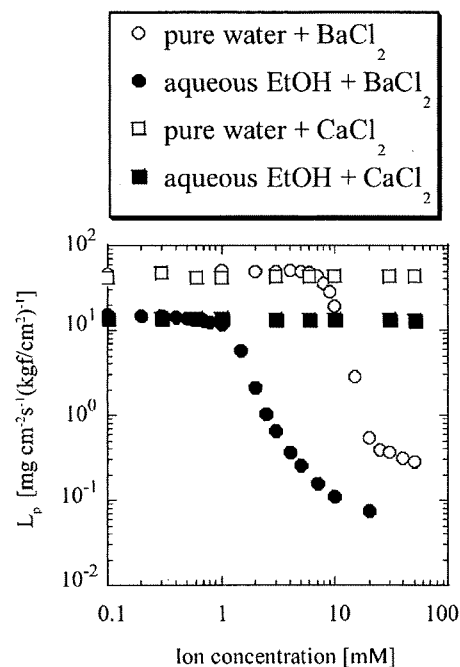


Fig. 11. Ion concentration dependence of the permeability coefficient of aqueous ethanol solutions (19.6 mol%) or water (0 mol%) containing BaCl_2 or CaCl_2 through a molecular recognition ion gating membrane. Temperature was 39.5 °C for the 19.6 mol% aqueous solution and 27.3 °C for pure water.

be applied to the gating membrane for sensing materials in the engineering field in future.

In terms of the phase diagram of Fig. 4, the globule-state region at around 15 mol% of ethanol is a quite sensitive region to respond to ion signals. These phenomena can probably be explained in terms of the complex formation constant of the crown ether moiety of BCAM in the grafted copolymer. Complex formation constants of benzo[18]crown-6 with inorganic ions have been previously reported to increase with increasing methanol mole ratio in aqueous methanol solution (Log K between benzo[18]crown-6 and K^+ : 1.84, 3.82, 4.75 and 5.29 at 0, 70, 80, 90 and 100 wt% of methanol [30]). We also reported that the complex formation constant of the crown ether moiety of BCAM increased with increasing methanol mole ratio in aqueous methanol solution (Log K between BCAM and K^+ : 1.0, 2.2, 2.9 and 3.5 at 0, 70, 80, 90 and 100 wt% of methanol [29]). Although detailed data on the complex formation constants of benzo[18]crown-6 in aqueous ethanol solution have not been reported to our knowledge, the tendency in the case of ethanol can reasonably be assumed to be similar to that in the case of methanol. In addition, the sensitivity enhancement of the crown ether receptors occurred by the increase in complex formation constant with increasing ethanol ratio.

We can understand the sensitivity enhancement roughly as described above, but the detailed mechanism needs to be investigated more deeply. The mechanism for the reentrant phase transition of poly-NIPAM is not yet clear, because water, ethanol and poly-NIPAM interact with each other in a complicated manner; thus, the effect of complexation between BCAM and a signal ion on the reentrant phase transition at the LCSTs is complicated, and further research is required.

4. Conclusions

We investigated both the reentrant phase transition of linear poly-NIPAM-co-BCAM and the response of a molecular recognition

ion gating membrane to signal ions in aqueous ethanol solutions. The reentrant phase transition of poly-NIPAM-co-BCAm showed LCST/LCST-type behavior in ethanol, which is different from the previous report of poly-NIPAM. In addition, the phase diagram of poly-NIPAM-co-BCAm changed because of the stimuli of the ion signal. According to this unique reentrant phase transition behavior of poly-NIPAM-co-BCAm, the gating membrane repeatedly and stably responded to ion signals in the mixed solvent of ethanol and water, because the gating membrane was prepared using synthetic polymers only. We also found that sensitivity and selectivity of the gating membrane to ion species changed in aqueous ethanol solution and, in particular, the sensitivity was enhanced by the addition of ethanol, which can be triggered by the increase of complex formation constant with increasing ethanol mole ratio in the solution.

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