Inter/Intramolecular Interaction and Chiral Recognition of Water-Soluble Copolymers and Their Hydrogels Containing an Optically Active Group

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Inter/Intramolecular Interaction and Chiral Recognition of Water-Soluble Copolymers and Their Hydrogels Containing an Optically Active Group

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Aqueous solutions of copolymers, consisting of *N*-isopropylacrylamide (NIPA) and optically active *N*-acryloyl-L-alanine (NALA) (poly(NIPA-*co*-NALA)), exhibited phase-separation temperatures (cloud point, T_c), and they were soluble at the lower temperatures and phase-separated at the higher temperatures. The T_c decreased with increasing NALA composition and increased with increasing pH of the solutions. The results of T_c measurements in the presence of NaCl and a urea derivative and Fourier transform infrared measurements with a curve-fitting method indicated that an increase in the composition of NALA in the copolymers induced hydrogen bonding in and between the copolymer chains. The partition coefficient (K) and diffusion coefficient (D) of *R*- and *S*-[1-(dimethylamino)ethyl]ferrocene (DMAEFc) in poly(NIPA-*co*-NALA) gels in aqueous solutions were estimated by a membrane permeation method. Negligible difference was observed for K between *R*- and *S*-DMAEFc; however, D of *R*-DMAEFc became smaller than that of *S*-DMAEFc when the gels started to collapse.

Introduction

Biopolymers such as proteins are folded into unique conformations when they exhibit their specific functions, such as molecular recognition and catalytic activities. This particular conformation is known as the "folding state" of the proteins.[1] In the folding state, the protein chains are highly packed by interactions such as hydrogen bonding, electrostatic, and hydrophobic interactions to form the molecular recognition and catalytic sites.[1] However, the proteins are easily denatured to lose their specific functions with changes in temperature, ionic strength, and solvent polarity and with additives such as urea derivatives, while the denatured proteins can recover to the native conformation under suitable conditions to exhibit their original functions.[1] On the other hand, for synthetic molecules (macromolecules) to have molecular recognition ability, it has been believed to be essential that they have molecular cavities, which have minimum freedom of conformational change and fit guest molecules, as seen in host–guest chemistry[2] and in molecular imprinting chemistry.[3–5] Prereduced entropy of the host molecules has been considered to favor the formation of host–guest complexes.

Water-soluble polymers and their gels, which abruptly change their conformation and volume in response to a small change in solvent composition, pH, temperature, and light, have been recognized as intelligent materials.[6–15]

In response to external stimuli, these polymers interconvert between a solvent-swollen random coil and a solvent-expelled globule, and their gels interconvert between a swollen phase and a shrunken phase. There have been several studies to find an analogy between the folding state of proteins and the solvent-expelled globule or shrunken phase of the water-soluble polymers and their gels.[16,17] It is quite interesting to explore hydrogels that undergo large conformation changes during swelling change but still exhibit molecular recognition ability in their shrunken states.[18]

In the water-soluble polymers and their gels, much interest has been paid to poly(*N*-isopropylacrylamide) (poly(NIPA)). Poly(NIPA) is a thermosensitive water-soluble polymer and is soluble in water as random coils at low temperatures, but it becomes insoluble above ca. 33 °C, that is, T_c.[19] Corresponding to this phase separation, cross-linked poly(NIPA) gels undergo a volume–phase transition.[8,20,21] Copolymers consisting of NIPA and various functional monomers exhibit diverse functions, de-

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pending on the properties of the combined comonomers.[22,23] It was found that NIPA/acrylic acid copolymer hydrogels, prepared in the presence of a guest molecule in a nonpolar solvent, could undergo a large swelling change (conformational change) but still retain molecular recognition ability in the shrunken states, as indicated by the specific volume change in response to the concentration of the guest molecule.[24]

In this study, novel water-soluble chiral copolymers containing NIPA and their hydrogels were prepared and characterized. *N*-Acryloyl alanine was selected as a chiral comonomer. The response of the copolymers toward temperature, pH, and additives indicated much stronger inter- and intramolecular hydrogen bonding interaction compared to that of poly(NIPA), which was also supported by Fourier transform infrared (FT-IR) spectra. Diffusion and partition coefficients of chiral compounds in the cross-linked chiral gels were explored as a function of temperature using a membrane permeation method, focusing on the change in the environment of the guest molecules in the hydrogels with temperature.

Experimental Section

Monomer Synthesis. NIPA, courtesy of Kojin Co., Japan, was purified by recrystallization in a toluene/hexane mixture. *N*-acryloyl-L-alanine (NALA), *N*-acryloyl-D-alanine (NADA), and *N*-acryloyl-D,L-alanine (NARA) were synthesized from acryloyl chloride and L-alanine, D-alanine, and D,L-alanine, respectively. The synthesis of these monomers is described elsewhere.[25–27]

Mass spectra (JEOL JMS-AX500), differential scanning calorimetry (Seiko Instruments DSC 220C), ^{1}H NMR, ^{13}C NMR (JEOL FX-270), and FT-IR (Perkin-Elmer Spectrum 1000) measurements were carried out to characterize the monomers. Circular dichroism (CD, JEOL J-725) spectra were measured to identify the optical property at a monomer concentration of 0.1 mM. Mass-spectra (CI mode): NALA $(M + 1) = 144$, NADA $(M + 1) = 144$, NARA $(M + 1) = 144$ $(M = 143$ calculated for C_6H_9-NO_3). Melting point: NALA $= 161.2$ °C, NADA $= 164.9$ °C, NARA $= 135.2$ °C (lit.:[25–27] NALA, NADA $= 161$ °C; NARA $= 135$ °C). ^{1}H NMR (270 MHz, DMSO-d_6, δ from TMS): NALA δ $= 1.29$ (d, $J = 7.3$ Hz, 3H), 4.27 (m, 1H), 5.60 (dd, $J_1 = 2.3$ Hz, $J_2 = 10.2$ Hz, 1H), 6.09 (dd, $J_1 = 2.3$ Hz, $J_3 = 17.3$ Hz, 1H), 6.29 (dd, $J_2 = 10.2$ Hz, $J_3 = 17.3$ Hz, 1H), 8.40 (d, 1H), 12.6 (s, 1H); NADA δ $= 1.29$ (d, $J = 7.3$ Hz, 3H), 4.27 (m, 1H), 5.60 (dd, $J_1 = 2.3$ Hz, $J_2 = 10.2$ Hz, 1H), 6.09 (dd, $J_1 = 2.3$ Hz, $J_3 = 17.3$ Hz, 1H), 6.29 (dd, $J_2 = 10.2$ Hz, $J_3 = 17.3$ Hz, 1H), 8.40 (d, 1H), 12.6 (s, 1H); NARA δ $= 1.29$ (d, $J = 7.3$ Hz, 3H), 4.27 (m, 1H), 5.60 (dd, $J_1 = 2.3$ Hz, $J_2 = 10.2$ Hz, 1H), 6.10 (dd, $J_1 = 2.3$ Hz, $J_3 = 17.3$ Hz, 1H), 6.29 (dd, $J_2 = 10.2$ Hz, $J_3 = 17.3$ Hz, 1H), 8.40 (d, 1H), 12.6 (s, 1H). ^{13}C NMR (67.9 MHz, DMSO-d_6, δ from TMS): NALA δ $= 17.3$, 47.6, 125.8, 131.4, 164.3, 174.2; NADA δ $= 17.3$, 47.6, 125.8, 131.4, 164.3, 174.2; NARA δ $= 17.3$, 47.6, 125.8, 131.4, 164.3, 174.2. IR (KBr, cm^{-1}): NALA N–H (3350), C=C–H (3050), C–H (2970), C=O(amide) (1640, 1545), C=O(acid) (1725); NADA N–H (3330), C=C–H (3050), C–H (2970), C=O(amide) (1645, 1545), C=O(acid) (1725); NARA N–H (3300), C=C–H (3050), C–H (2970), C=O(amide) (1645, 1540), C=O(acid) (1725).

Linear Copolymer Synthesis. Optically active copolymers were synthesized using NIPA and NALA (Figure 1). Various compositions of the monomers, NIPA and NALA, were dissolved in a given amount of water, and the total monomer concentration was kept constant at 1.33 M. Feed compositions for the copolymers are summarized in Table 1. The monomer solutions were bubbled with N_2 gas for 30 min at 0 °C. Free-radical polymerization was
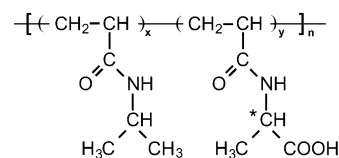
Figure 1. Structure of poly(NIPA-*co*-NALA).

Table 1. Characterization of Poly(NIPA-*co*-NALA)

	poly(NIPA-*co*-NALA, x/y)^a					
	20/0^b	19/1^c	18/2^c	17/3^c	16/4^c	15/5^c
feed composition of [NIPA]/[NALA]	20:0	19:1	18:2	17:3	16:4	15:5
conversion (%)	15.4	12.5	9.5	12.7	13.6	6.7
copolymer composition of [NIPA]/[NALA]	20:0	19:1	18:2	17:3	16:4	15:5
M_w	82000	93900	77600	66500	63900	57600
M_n	26800	86700	71000	58300	60900	53800
M_w/M_n	3.1	1.1	1.1	1.1	1.1	1.1

^{a} Dialyzed with cellulose dialysis membrane. ^{b} Molecular weight cutoff of cellulose dialysis membrane $= 8000$. ^{c} Molecular weight cutoff of cellulose dialysis membrane $= 12\,000$.

performed at 4 °C for 24 h using 0.35 mol % ammonium persulfate as an initiator and sodium metabisulfate as an accelerator. Purification was carried out by dialyzing against distilled water and then a diluted HCl aqueous solution to completely convert the copolymers into an acidic form.[28] Dialyzed solutions were lyophilized and finally dried under reduced pressure with P_2O_5 at room temperature for 48 h. In the same way, the D-form and the racemic form of the copolymers containing NADA and NARA were prepared.

Characterization of Copolymers. The copolymer compositions, number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (M_n/M_w) were determined from ^{1}H NMR and gel permeation chromatography (GPC, Shimadzu LC-10), respectively. *N,N*-Dimethyl formamide was used for the elution solvent of GPC, and the GPC columns were calibrated by polystyrene standards (Toso). CD spectra were measured to confirm the optical property of copolymer aqueous solutions at a concentration of 0.5 mM based on the monomer.

T_c Measurements. The T_c of NIPA/NALA copolymer aqueous solutions (1 wt %) was measured using a UV–vis spectrometer (Shimadzu UV-2500C) at a fixed wavelength (500 nm) with a water-jacketed cell holder coupled with a temperature-controlled circulating bath to determine the turbidity of the polymer solution as a function of temperature. The temperature was raised at a rate of 0.4 °C/min. The temperatures at 50% light transmittance of the copolymer solutions were defined as the T_c.[20]

Potentiometric Titration. Poly(NIPA-*co*-NALA, 16/4) and NALA aqueous solutions were prepared at a concentration of 2.52×10^{-5} M carboxyl groups. These solutions were titrated by a 0.1 M NaOH aqueous solution at 20 °C with recording the pH (Toa Electronics HM-30V) of the solutions.

FT-IR Measurements. FT-IR spectra of the copolymer solutions were obtained on a Perkin-Elmer FT-IR Spectrum 1000 spectrophotometer at room temperature (ca. 20 °C). The two bands were selected as characteristic absorptions to reveal hydrogen bonds in the copolymers: stretching vibration modes of C=O in amide groups ($\nu_{C=O(amide)}$) and in carboxyl groups ($\nu_{C=O(acid)}$). These absorptions appear in a region from 1500 to 1800 cm^{-1}, where H_2O also shows a strong absorption. Thus, D_2O, which shows a weak absorption around 1600 cm^{-1}, was used as a solvent for the IR measurements. The copolymers were dissolved in D_2O and held at 4 °C for 2 days. A 5 wt % copolymer solution was put between two silicon plates (courtesy of Mitsubishi Silicon Materials Co., Japan) separated by a spacer of thin poly(ethylene terephthalate) film (50 μm thick). For each spectrum, 512 scans with a resolution of 2 cm^{-1} were recorded for the copolymer solutions. The spectra were corrected for the D_2O contribution by subtraction of the D_2O spectrum recorded under the same conditions.

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Synthesis and Characterization of Poly(NIPA-*co*-NALA, 16/4) Gel. NIPA, NALA, and a cross-linking agent, *N*,*N*-methylenebisacrylamide (Bis), were dissolved in a given amount of water at a monomer ratio of NIPA/NALA/Bis = 16:4:1, and the total monomer concentration was kept constant at 1.33 M. The solution was bubbled with N_2 gas for 30 min at 0 °C. Free-radical polymerization was performed at 4 °C for 24 h using 0.35 mol % ammonium persulfate as an initiator and sodium metabisulfate as an accelerator. Polymerization was carried out in a sample tube in which micropipets with an inner diameter of 145 μm were immersed. Rodlike gels were taken out from the pipets. The obtained gels were washed in distilled water at 4 °C and immersed in a benzoate buffer solution (10 mM, pH = 3.5, I = 50 mM with NaCl). The gel composition was determined from ^{1}H NMR. The volume change of the gels as a function of temperature was determined using an optical microscope (Nikon TMS-F) and was expressed by the swelling ratios of the gels (V/V_0), where V is the volume of gels under a certain condition and V_0 is the volume of gels at the preparation.

Membrane Permeation Method. Polymer gels for this experiment were prepared in the same way as described above. The polymerization was carried out in a cell consisting of two glass plates with a poly(tetrafluoroethylene) spacer (1 mm thick). The obtained slab-gels were washed in distilled water at 4 °C and immersed in the benzoate buffer solution at 4 °C. After 5 days, the temperature of the slab-gel in the buffer was raised to 20 °C with a heating rate of 1 °C/h and kept at 20 °C for a day. The gel was punched out to disks of a suitable size (ca. 2.5 cm diameter) and sandwiched between two double-jacketed temperature-controlled 10 mL cells (see Figure 9). The temperature of the buffer containing the disk gels was raised to 25 °C with a heating rate of 1 °C/h and kept at 25 °C for a day, and the membrane was set in the cells. In the same manner, the gel membrane at 30 °C was prepared. The benzoate buffer solutions kept at 20, 25, and 30 °C were poured into the cell and held for 30 min, respectively. Then, a small amount of the buffer solution in the donor cell was sucked up. A 1 mL portion of 13 mM *R*- or *S*-[1-(dimethylamino)ethyl]ferrocene (DMAEFc) in the buffer solution held at each temperature was added to the donor cell, and the cell was filled up to 10 mL with the buffer. We defined this moment as the starting time ($t = 0$) of the permeation experiments. A 100 μL portion of the solution in the acceptor cell was successively collected with time. The concentration of the probe molecule, DMAEFc, in this sample solution was detected by using high-performance liquid chromatography (Shimadzu LC-10) with a UV–vis detector.

Results and Discussion

Characterization of Copolymers. ^{1}H NMR spectra were measured to identify the structure of copolymers and the copolymer compositions. ^{1}H NMR (270 MHz, DMSO-d_6, δ from TMS): δ = 1.10 (CH$_3$– in NIPA), 1.25 (CH$_3$– in NALA, NADA, and NARA), 1.40 (–CH$_2$– in the main chain), 1.95 (>CH– in the main chain), 3.85 (>CH– in NIPA), 4.20 (>CH– in NALA, NADA, and NARA), 7.30 (–CO–NH–). By using the integrated signal ratios at 3.85 and 4.20 ppm, the copolymer compositions were determined. The conversions, copolymer compositions, M_n, M_w, and M_w/M_n of poly(NIPA-*co*-NALA) are summarized in Table 1. The M_w/M_n values of the copolymers are close to 1.0, which is unusual for free-radical polymerization. This seems to be caused by the dialysis with a dialysis membrane with a molecular weight cutoff of 12 000. The monomer compositions in the feed for preparation and the copolymer compositions coincide with each other, indicating that the monomer reactivity of NIPA and NALA is similar. This is reasonable, because both of the monomers are acrylamide derivatives. These copolymers have a random nature in terms of the monomer sequence.

The CD spectra of NALA and NADA were symmetrically inverted, whereas the NARA and NADA = 1:1 mixture did not exhibit CD (Supporting Information). Poly(NIPA) and poly(NIPA-*co*-NARA) did not exhibit CD. On the other
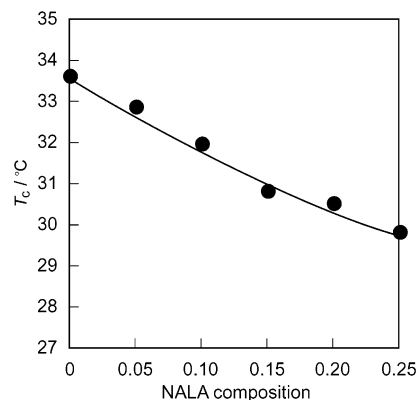
Figure 2. T_c for 1 wt % aqueous solutions of poly(NIPA-*co*-NALA) as a function of NALA composition.

hand, the NALA and NADA copolymers exhibit symmetric CD spectra, and their magnitude depends on their compositions. It was evident that poly(NIPA-*co*-NALA) and poly(NIPA-*co*-NADA) are optically active and their CD is symmetrical (Supporting Information).

T_c of Copolymers. Aqueous solutions of poly(NIPA-*co*-NALA), poly(NIPA-*co*-NADA), and poly(NIPA-*co*-NARA) are thermosensitive and exhibit T_c; that is, they are soluble at the lower temperatures and phase-separated at the higher temperatures. No difference was observed in the T_c, depending on the optical activity of the acryloyl alanine monomers. Figure 2 shows T_c of poly(NIPA-*co*-NALA) aqueous solutions as a function of NALA composition in the copolymers. T_c shifts to lower temperatures with increasing composition of NALA (vide infra).

Potentiometric titration measurements were made to know the pK_a of NALA and poly(NIPA-*co*-NALA, 16/4) as a function of the degree of dissociation (α) (Figure 3a).[28] The pK_a of NALA is constant (pK_a = 3.5), independent of α. On the contrary, pK_a of poly(NIPA-*co*-NALA, 8/2) is higher than that of NALA and increases with increasing α. This kind of behavior is common for polyelectrolytes of weak acids, as seen in poly(acrylic acid) and poly-(methacrylic acid).[29] Figure 3b shows the effect of pH on the T_c of aqueous solutions of poly(NIPA-*co*-NALA, 16/4). It can be seen that the T_c of the copolymer sharply increases with increasing pH of the solutions. The T_c could not be detected at pH = 5.0 by a temperature rise up to 60 °C. It is found that the T_c of poly(NIPA-*co*-NALA) depends on the pH of the solutions as well as NALA composition (Figure 2).

The T_c of NIPA copolymers shifts to higher temperatures by introducing hydrophilic comonomers in many cases, because hydrophobic interaction is the main reason for the phase separation of these polymers.[30–33] It had been supposed that the T_c of poly(NIPA-*co*-NALA) aqueous solutions shifted to higher temperatures with increasing NALA composition, since NALA had a hydrophilic moiety, the carboxyl group. However, the T_c becomes lower with increasing NALA composition in the copolymers (Figure 2). To elucidate the dominant reason for this behavior, T_c values of poly(NIPA) and poly(NIPA-*co*-NALA, 16/4) were measured and compared under different conditions.
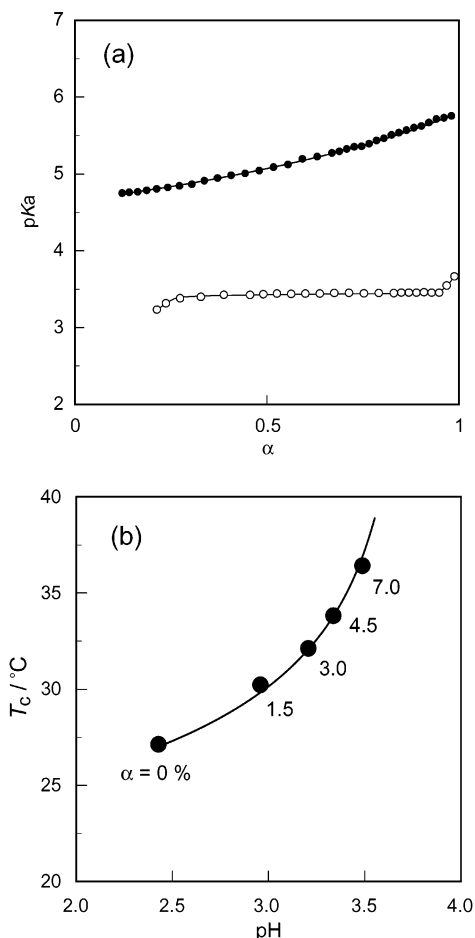
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Figure 3. (a) pK_a plotted against α (degree of dissociation) for poly(NIPA-*co*-NALA, 16/4), ●, and NALA, ○. (b) T_c for 1 wt % aqueous solutions of poly(NIPA-*co*-NALA, 16/4) as a function of the pH of the solutions.

In the presence of NaCl, the T_c of poly(NIPA-*co*-NALA, 16/4) aqueous solutions shifts to lower temperatures with increasing NaCl concentration, in the same manner as poly(NIPA) aqueous solutions behave (Figure 4a). The slopes of the T_c versus NaCl concentration are similar in both of the polymers. The effect of the addition of NaCl, causing decreases in the T_c, is interpreted in terms of its strong ionic hydration. The strong hydration removes water from the polymer chains, which leads to dehydration and aggregation of the polymer chains. This effect lowers the phase transition temperatures.

On the other hand, in the presence of a urea derivative, 1,1-dimethylurea, the phase separation behaviors of poly-(NIPA) and poly(NIPA-*co*-NALA, 16/4) are different (Figure 4b). The T_c of poly(NIPA-*co*-NALA, 16/4) shifts to higher temperatures with an increase in 1,1-dimethylurea concentrations. The shift is much larger than that of poly-(NIPA). Urea derivatives are very liable to make hydrogen bonds so that the addition of 1,1-dimethylurea is likely to destroy most of the hydrogen bonds existing in and between the polymer chains. A decrease in the number of the hydrogen bonds may weaken the cohesive force of the polymer chains, resulting in a shift of the T_c to higher temperatures. A modest increase in the T_c of poly(NIPA) with the addition of 1,1-dimethylurea seems to be caused by destroying the hydrogen bonds formed between the amide groups in the polymer chains. A relatively large change in the T_c of poly(NIPA-*co*-NALA, 16/4) means that 1,1-dimethyl urea destroys not only hydrogen bonds between amide groups but also hydrogen bonds between
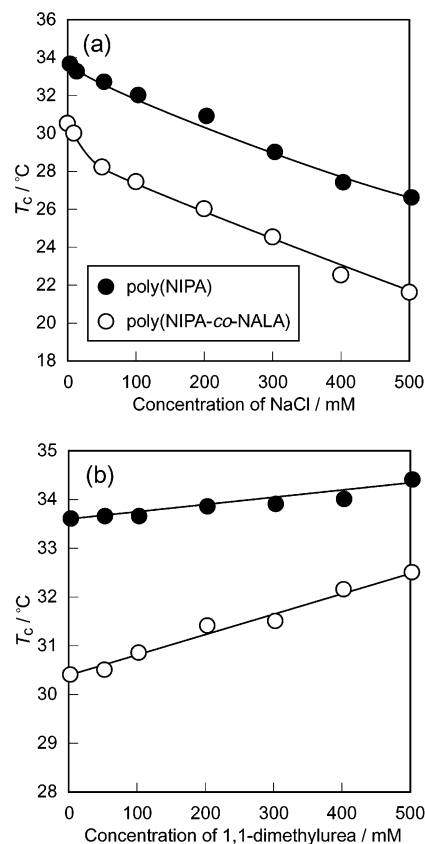
Figure 4. T_c for 1 wt % aqueous solutions of poly(NIPA) and poly(NIPA-*co*-NALA, 16/4) as a function of NaCl concentration (a) and 1,1-dimethylurea concentration (b).
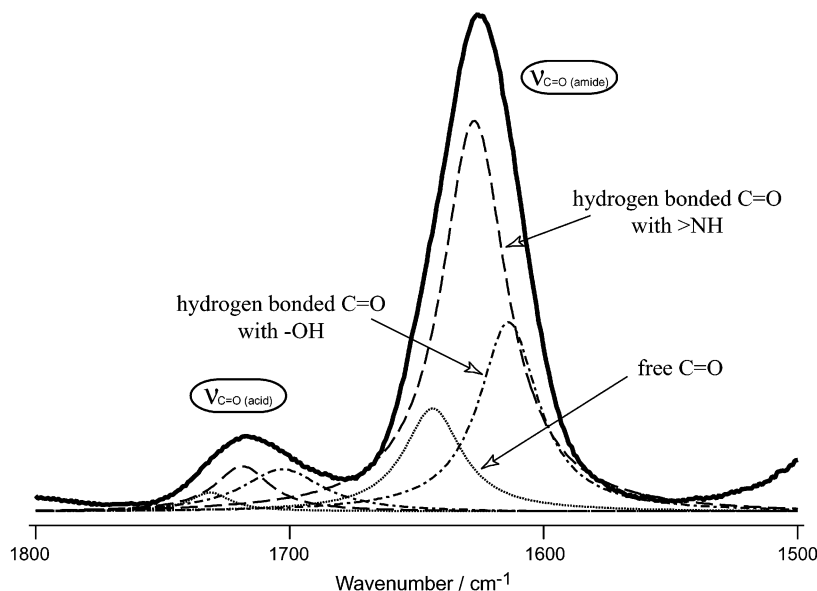
amide groups and NALA carbonyl groups. It seems to be reasonable to consider that carboxyl groups in the copolymer have a strong ability to make hydrogen bonds in and between the polymer chains, which strengthens the cohesive energy of the polymer chains, leading the T_c to lower temperatures with increasing NALA composition.

Intra- and Intermolecular Interactions of Poly-(NIPA-*co*-NALA) in Aqueous Solutions. IR spectroscopy is a useful technique to obtain information about inter/intramolecular interactions, especially hydrogen bonds between the molecules, since IR measurements can detect the molecular vibrations affected by hydrogen bonds. Figure 5 shows a representative IR spectrum of poly(NIPA-*co*-NALA, 16/4). The stretching vibrations of amide carbonyl groups (amide I band, $\nu_{C=O(amide)}$) and carboxyl groups ($\nu_{C=O(acid)}$) are observed at 1624 and 1717 cm^{-1}, respectively.[34−36] When hydrogen bonds are formed, the wavenumber of the stretching vibrations is expected to shift to a lower value. From the spectrum, however, it cannot be interpreted whether the hydrogen bonds exist or not. Accordingly, the components of each carbonyl stretching in the IR spectra are deconvoluted by a curve-fitting program, on the assumption that the peak shape of the components is Lorentzian. While the baseline and the peak shape were fixed, an iterative procedure was employed to obtain the best fit, without changing the wavenumber of each component.[37,38] Although two-, three-, and four-component fittings were conducted, the three-component fitting gave the best results. Thus, each

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$v_{C=O \text{ (amide)}}$

hydrogen bonded C=O
with >NH

hydrogen bonded C=O
with -OH

free C=O

$v_{C=O \text{ (acid)}}$

Wavenumber / cm^{-1}

1800   1700   1600   1500

Figure 5. FT-IR spectrum and its deconvolution results for a 5 wt % D$_2$O solution of poly(NIPA-*co*-NALA, 16/4).

Table 2. Assignment of Curve-Fitting Results of Carboxyl Bands ($v_{C=O(acid)}$) and Amide I Bands ($v_{C=O(amide)}$) for Poly(NIPA-*co*-NALA)

$v_{C=O \text{ (acid)}}$ / cm^{-1} ... $v_{C=O \text{ (amide)}}$ / cm^{-1}

Hydrogen Bonded with -OH groups: 1702 ... 1614

Hydrogen Bonded with >NH groups: 1719 ... 1628

Free: 1732 ... 1642

carbonyl vibration is considered to consist of three components: hydrogen-bonded with −OH groups, hydrogen-bonded with >NH groups, and free carbonyl groups (Table 2).[39,40] Actually, −OH and >NH in Table 2 mean −OD and >ND, respectively, since we used D$_2$O as a solvent so that deuterium exchange occurred.[41,42]

Figure 6 shows deconvolution results of C=O$_{(amide)}$ and C=O$_{(acid)}$ bands for the copolymers as a function of NALA composition. It can be seen from Figure 6 (bottom) that the C=O$_{(amide)}$ groups of poly(NIPA) are equally hydrogen-

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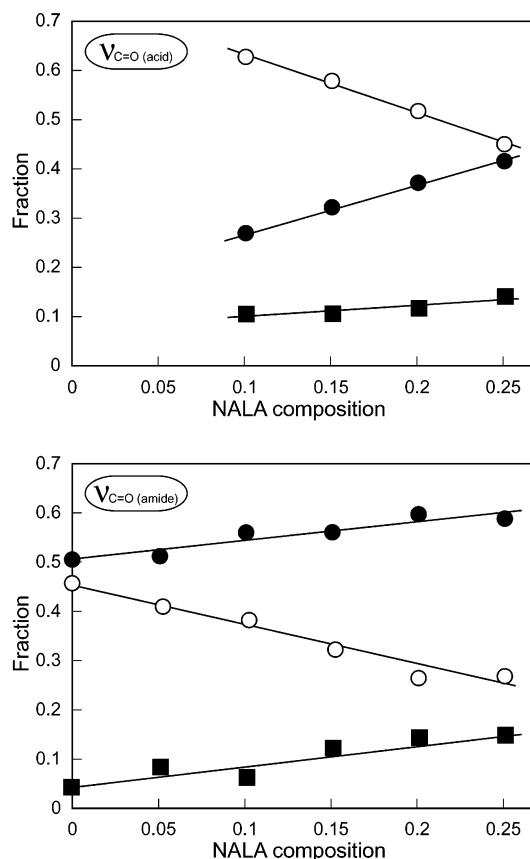(38) High, M. S.; Painter, P. C.; Coleman, M. M. *Macromolecules* **1992**, *25*, 797.
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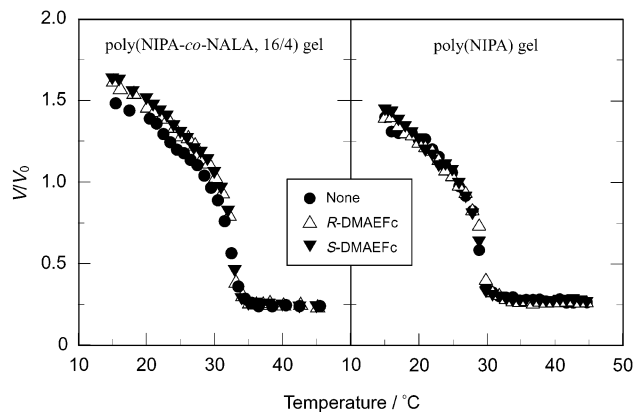(42) Scarpa, J. S.; Mueller, D. D.; Klotz, I. M. *J. Am. Chem. Soc.* **1967**, *89*, 6024.

Figure 6. Deconvolution results for the FT-IR spectra of a 5 wt % D_2O solution of poly(NIPA-*co*-NALA) as a function of NALA composition: ○, hydrogen-bonded with −OH groups; ●, hydrogen-bonded with >NH groups; ■, free.

bonded with −OH and >NH groups. With increasing NALA compositions, the former decreases, and the latter increases. As for $C=O_{(acid)}$ groups, the majority (ca. 60%) are hydrogen-bonded with −OH groups when the NALA composition is 0.1. With increasing the NALA composition, $C=O_{(acid)}$ groups hydrogen-bonded with −OH groups largely decrease, while those hydrogen-bonded with >NH groups symmetrically increase. Since it can be supposed that the largest part of −OH groups involved in the hydrogen bonding is that of the solvent (deuterium oxide) in such aqueous solutions, these results reveal that hydrated carbonyl groups decrease with increasing NALA composition and that hydrogen bonding in and between the polymer chains is enhanced.

The fact that the pK_a of poly(NIPA-*co*-NALA) is ca. 5.0, despite the pK_a of NALA being 3.5, suggests that the hydrophobic environment, resulting from hydrogen-bonded amide groups and isopropyl groups around the carboxyl groups, is one reason which prevents dissociation of the carboxyl groups. It is reported that the T_c of NIPA copolymers, which have carboxyl groups, shifts to lower temperatures when the pH of the solution is reduced.[43,44] The primary reason for these phenomena is a decrease in the dissociation ability, due to hydrogen bonds. The carboxyl groups in poly(NIPA-*co*-NALA) are mainly hydrated by water when the NALA composition is low, but the hydrogen bonds with water molecules are replaced by those with >NH groups with increasing NALA composition. This is why the hydrogen-bonded species with

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Figure 7. Swelling ratios of poly(NIPA-*co*-NALA, 16/4) gel and poly(NIPA) gel in a benzoate buffer solution (pH = 3.5) in the absence and presence of *R*- or *S*-DMAEFc.

>NH groups increase with increasing NALA composition, as shown in Figure 6 (top).

From the T_c and IR measurements, we found that the introduction of NALA into poly(NIPA) intensified the ability of hydrogen bonding in and between the copolymer chains, so that T_c was lowered. An important interaction for the phase separation of poly(NIPA) from aqueous solutions is hydrophobic interaction between the isopropyl groups. In the case of poly(NIPA-*co*-NALA), a locally low-polar region, consisting of the isopropyl group and hydrogen-bonded amide/carboxyl groups, seems to appear near the T_c. It is presumed that the chiral moiety of NALA is concentrated in this hydrophobic and low-polar environment. This environment near the T_c may be specific in contrast to that in the random coil state.

Chiral Specificity for Membrane Permeation through Poly(NIPA-*co*-NALA) Gels. To discriminate the behavior of chiral molecules interacting with the copolymers, the polymer chains were immobilized by a cross-linker. Partition and diffusion coefficients of a chiral probe in the polymer network can be estimated from elution profiles of the probe passing through the gel membranes. *R*- and *S*-DMAEFc were selected as the probe, because they have a hydrophobic moiety as well as a basic moiety. For the sake of solubility of the probe molecules, the benzoate buffer solution (pH = 3.5) was used.

Figure 7 shows the correlation between V/V_0 and the temperature of poly(NIPA-*co*-NALA, 16/4) gel and poly-(NIPA) gel as a reference in the absence and presence of *R*- or *S*-DMAEFc. It is hard to say that meaningful differences are observed in the swelling ratios in the absence and the presence of *R*- or *S*-DMAEFc for both the poly(NIPA-*co*-NALA, 16/4) and poly(NIPA) gels.

Figure 8 shows typical membrane permeation results. Because gels consist of three-dimensional networks and solutions, probe molecules can diffuse through the gel membranes. The motive force of the passive diffusion is a concentration difference of the probe molecules between the two compartments. Based on the random walk model, the diffusion coefficient, *D*, of the probe molecule in the gel can be expressed by[45]
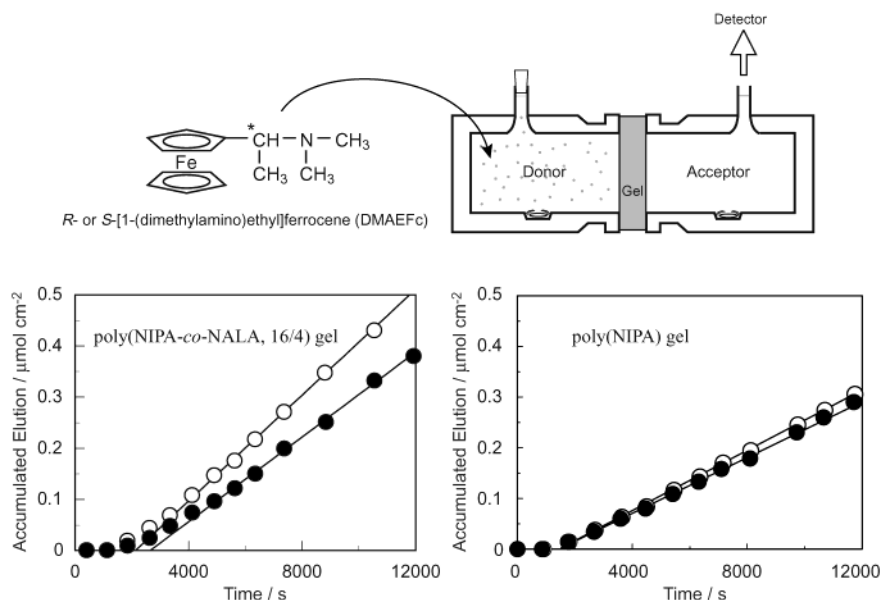
$$D = \frac{d^2}{6\tau} \tag{1}$$

where *d* is the thickness of the gel membrane and τ is the necessary time to pass the probe molecule through the gel

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Figure 8. Elution time courses of *R*-DMAEFc, ●, and *S*-DMAEFc, ○, through poly(NIPA-*co*-NALA, 16/4) gel and poly(NIPA) gel immersed in a benzoate buffer solution (pH = 3.5) at 25 °C.

(lag time). τ can be determined from the intercept by extrapolating a time-accumulative permeation curve at steady state[45] (see Figure 8). The flux, *J*, of the probe molecule due to the diffusion can be expressed by

$$J = -D\frac{dC}{dx} \tag{2}$$

dC/dx means the concentration gradient of the probe molecule in the membrane. The concentration of the probe molecule at the donor surface of the gel is defined as C^*. If the concentration of the probe molecule in the donor cell (C_0) is assumed to be constant and that in the acceptor cell is approximately 0, eq 2 can be rewritten as follows:

$$J = -D\frac{dC}{dx} = D\frac{C^*}{d} = D\frac{C^*}{C_0}\frac{C_0}{d} = DK\frac{C_0}{d} \tag{3}$$

where *K* is the partition coefficient ($K = C^*/C_0$) of the probe molecule into the gel membrane. Thus, *D* and *K* of the probe molecule in the gel membranes can be determined from the permeation experiments as shown in Figure 8 and eqs 1 and 3.

Appreciable differences are observed between the permeation behavior of *R*- and *S*-DMAEFc through the poly(NIPA-*co*-NALA) gel membrane, whereas negligible difference is observed for the poly(NIPA) gel membrane, as can be seen from Figure 8. Figure 9 shows *D* and *K* of *R*- and *S*-DMAEFc in the poly(NIPA-*co*-NALA, 16/4) and poly(NIPA) gel membranes at different temperatures. When the poly(NIPA-*co*-NALA, 16/4) gel was completely shrunken, for example, at 35 °C, the permeation experiments were not successful, because the membranes were prone to breakage during the experiments. For the completely shrunken poly(NIPA) gel membrane at 30 °C, the concentration of the probe molecule in the acceptor cell fell below the measurable limits. When a comparison is made between the permeation results of the poly(NIPA-*co*-NALA, 16/4) and poly(NIPA) gel membranes at the same temperature, *D* is larger for the poly(NIPA) gel membrane, and *K* is larger for the poly(NIPA-*co*-NALA, 8/2) gel membrane. Looking at the swelling ratios of poly-(NIPA) and poly(NIPA-*co*-NALA) gels, the swelling ratios at the same temperature are larger for the latter in this
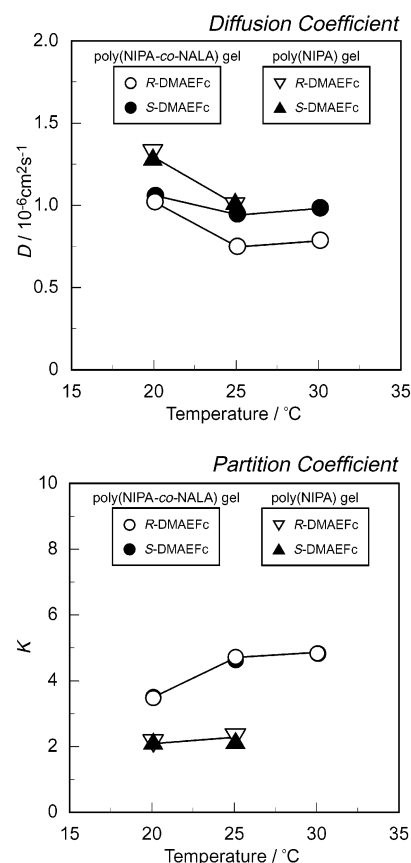
Figure 9. Temperature dependence of diffusion coefficients (*D*) and partition coefficients (*K*) of *R*- and *S*-DMAEFc in poly-(NIPA-*co*-NALA, 16/4) gel and poly(NIPA) gel immersed in a benzoate buffer solution (pH = 3.5).

buffer solution (Figure 7). This resulted from partial dissociation of the carboxylic groups of NIPA, and the α can be approximated as 0.07 at 20 °C in this buffer from Figure 3b. Nevertheless, *D* and *K* of the probe molecule are smaller and larger, respectively, for the poly(NIPA-*co*-NALA) gel. The introduction of NALA to poly(NIPA) greatly enhances the interaction between the probe molecule and the polymer chains, which may reflect the

enhanced hydrogen-bonding interaction in and between the copolymer segments in the gel, as indicated by the T_c and IR results, as well as the acid−base interaction between the probe molecule and the copolymer chain. Especially, K values far larger than unity for the poly-(NIPA-*co*-NALA) gel, which increases with temperature (shrinkage of the gel), indicate that the probe molecule is thermodynamically stable in the gel membranes, compared to the probe in the solution. The pK_a value of poly-(NIPA-*co*-acrylic acid) gels increases with temperature-induced shrinkage of the gels.[46] By analogy, the dissociation of NALA in the poly(NIPA-*co*-NALA) gel may also be suppressed with the temperature-induced shrinkage.

Chiral selectivity of the copolymer membrane should also be addressed. There is no significant difference in K between R- and S-DMAEFc at every temperature. On the other hand, D at 20 °C shows no differences; however, D of R-DMAEFc becomes smaller than that of S-DMAEFc at 25 and 30 °C. There is no difference in D between the enantiomers in the poly(NIPA) gel. Figure 7 indicates that the poly(NIPA-*co*-NALA, 16/4) gel is swollen at 20 °C and complete collapse of the gel starts at 30 °C. It can be understood that the chiral selectivity is emphasized when the gel starts to collapse. K is a static parameter, whereas

D is a dynamic parameter that can reflect the interaction between the polymer chain and the probe in each step of the diffusion process. For this reason, the chiral selectivity might be pronounced in the difference in D. In the copolymer gel, a low-polar region consisting of the isopropyl group and hydrogen-bonded amide/carboxyl groups is formed when approaching the T_c. The chiral moiety of NALA concentrated in this hydrophobic environment may be responsible for the chiral selectivity. Chiral selectivity of DMAEFc in this optically active gel has also been confirmed by using an electroanalytical method,[47,48] and the results will be published elsewhere.[49]

Supporting Information Available: Plots of $[\theta]$ versus wavelength for NADA, NALA, NARA, NALA + NADA, poly-(NIPA-*co*-NALA), and poly(NIPA-*co*-NADA). This material is available free of charge via the Internet at http://pubs.acs.org.

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