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# Dual-Stimuli-Responsive Block Copolymers Derived from Proline Derivatives

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ABSTRACT: Novel pH- and thermo-responsive block copolymers comprising proline-based segments were synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization. Poly(*N*-acryloyl-L-proline methyl ester), poly(A-Pro-OMe), was selected as a thermo-responsive segment, whereas poly(*N*-acryloyl-L-proline), poly(A-Pro-OH), was employed as a weak anionic polyelectrolyte. The chain extension from the dithiocarbamate-terminated poly(A-Pro-OH) to A-Pro-OMe could be well controlled under suitable conditions, and it provided block copolymers with as-designed chain structures and low polydispersities. The characteristic pH- and thermo-responsive properties of the block copolymers were then investigated in aqueous solutions. By varying the pH, salt concentration, and comonomer composition, the lower critical solution temperature (LCST) can be tuned between 18 and 55 °C. The chiroptical properties and assembled structures of the resulting block copolymers in aqueous solutions were evaluated by circular dichroism (CD) and dynamic light scattering (DLS) measurements.

## Introduction

Temperature- and pH-sensitive polymers and gels have recently attracted significant research interest because these dual functional systems have great importance in biological applications and can mimic the responsive macromolecules found in nature.[1−5] The aqueous solutions of these dual-stimuli-responsive materials and their swelling properties can abruptly and reversibly change in response to simultaneous pH and temperature changes; hence, such polymers have numerous potential applications such as controlled drug delivery and release. In particular, there is growing interest in developing temperature- and pH-sensitive block copolymers since the ability to respond to both stimuli offers an additional control over assembled structures, which is responses to combinations of external stimuli.[4,6−13]

The hierarchical structures of the stimuli-responsive block copolymers formed via self-assemblies are generally dependent on the structure and property of each segment, composition, and chain length, in addition to external stimuli such as pH, salt, and temperature. For the precise manipulation of the dual-responsive properties and stimuli-induced self-assembling behaviors, such as normal and reversible micelle formation, it is important to create block polymers composed of two stimuli-sensitive segments, each of which should possess narrow chain length distributions with controlled molar mass and composition.

The design and synthesis of novel block copolymers derived from amino acids are attracting significant attention because of their assembled structures through intra- and interchain associations via noncovalent forces and their potential applications as biodegradable and biomedical polymers.[11,14−19] In amino acid-based block copolymers, the nature of amino acid and the chirality and amphiphilicity encoded in their primary structures play crucial roles in determining their ordered structures and various functions. In recent years, considerable attention has been paid to developing novel amino acid-based block copolymers using controlled radical polymerization techniques.[14,20] Atom transfer radical polymerization,[20−27] nitroxide-mediated radical polymerization,[28,29] and reversible addition−fragmentation chain transfer (RAFT) polymerization[30−43] have been employed for the synthesis of a variety of peptide−polymer hybrids having well-defined structures and characteristic properties. In addition to the characteristic properties of the amino acid-containing segment, the composition of peptide−polymer hybrids provides a great opportunity to tune their chemical and physical properties; moreover, highly ordered structures can be obtained via the self-assembly process.

In this paper, we report the controlled synthesis of novel proline-based block copolymers having characteristic pH- and thermo-responsive properties by RAFT polymerization (Scheme 1). Amino acid-based block copolymers with dual-stimuli-responsive properties are of considerable scientific and industrial interest because these systems can be regarded as artificial and model proteins having stimuli-responsive properties; further, their repeating sequences hold and/or self-assemble into more highly ordered structures in response to pH and temperature. As a part of our continuous efforts to develop highly ordered amino acid-based architectures, we reported the controlled radical polymerization of acrylamides having various amino acid moieties in the side chains, including *N*-acryloyl-L-proline methyl ester (A-Pro-OMe),[41,42,44] *N*-acryloyl-L-phenylalanine methyl ester,[45] *N*-acryloyl-L-alanine,[46] *N*-acryloyl-L-phenylalanine,[47] *N*-acryloyl-L-proline (A-Pro-OH),[48] and *N*-acryloyl-4-*trans*-hydroxy-L-proline,[48] via the RAFT process. We also focused on thermo-responsive random and block copolymers involving A-Pro-OMe units, which exhibited a characteristic lower critical solution temperature (LCST) in aqueous solutions.[41,42] In this study, we prepared dual-stimuli-responsive block copolymers derived from the proline derivatives A-Pro-OH and A-Pro-OMe. L-Proline is the major constituent of collagen

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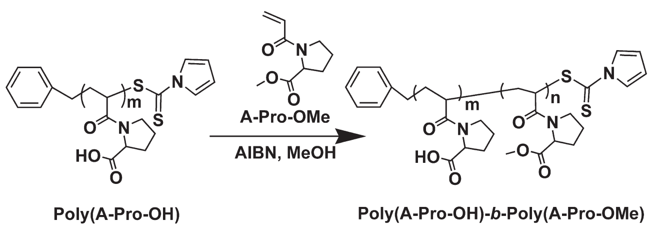
**Scheme 1. Synthesis of Block Copolymers: Reversible Addition–Fragmentation Chain Transfer (RAFT) Polymerization of *N*-Acryloyl-L-proline Methyl Ester (A-Pro-OMe) Using Poly(*N*-acryloyl-L-proline) (Poly(A-Pro-OH)) as a Macro-Chain-Transfer Agent**



and is responsible for various functions such as turn inducer in natural peptides and proteins[49,50] and for ordered structures such as the helix structure of oligoprolines.[51,52] Numerous studies on L-proline derivatives,[53,54] polymeric materials,[55] and their bio-related phenomena and applications[56,57] have been reported by many groups. In this study, poly(*N*-acryloyl-L-proline methyl ester), poly(A-Pro-OMe), was selected as a thermo-responsive segment, while poly(*N*-acryloyl-L-proline), poly(A-Pro-OH), was employed as a pH-responsive segment. Since A-Pro-OH has a carboxylic acid in the monomer unit, poly(A-Pro-OH) can be recognized as a weak polyelectrolyte, in which the degree of ionization is governed by the pH and ionic strength of the aqueous solution. Direct RAFT polymerization of these proline-containing monomers was carried out without protection chemistry in order to obtain block copolymers having low polydispersity and controlled chain length and composition.

## Experimental Section

**Materials.** 2,2′-Azobis(isobutyronitrile) (AIBN, Kanto Chemical, 97%) was purified by recrystallization from methanol. *N*-Acryloyl-L-proline methyl ester (A-Pro-OMe)[41,55] and *N*-acryloyl-L-proline (A-Pro-OH)[48,58] were prepared by the reaction of acryloyl chloride with the corresponding proline derivatives (L-proline methyl ester hydrochloride and L-proline, respectively) in accordance with the methods reported previously. The synthesis of benzyl 1-pyrrolecarbodithioate was conducted according to the procedure reported previously.[59,60] Methanol (dehydrated MeOH, Kanto Chemical, 99.8%) was used as received. The methylation agent, trimethylsilyldiazomethane (2 M solution in diethyl ether), was purchased from Aldrich and used as received. All the other materials were used without further purification.

**Synthesis of Block Copolymers.** All polymerizations were performed in a degassed sealed tube with AIBN as the initiator. A representative example of the synthesis of the block copolymer comprising A-Pro-OMe and A-Pro-OH is as follows: A-Pro-OH (1.20 g, 7.09 mmol), benzyl 1-pyrrolecarbodithioate (66.1 mg, 0.283 mmol), AIBN (9.3 mg, 0.056 mmol), and methanol (4.8 mL) were placed in a dry ampule, and the solution was then degassed using three freeze–evacuate–thaw cycles. After the ampule was flame-sealed under vacuum, it was stirred at 60 °C for 17 h. The reaction was stopped by rapid cooling with liquid nitrogen, and the monomer conversion (95%) was determined by the $^1$H NMR spectrum of the polymerization mixture in $CD_3OD$. After removing the solvents by evaporation, a part of the crude poly(A-Pro-OH) was modified by methylation using trimethylsilyldiazomethane, and the resulting poly(A-Pro-OMe) was used directly for size-exclusion chromatography (SEC) measurements without any purification (number-average molecular weight $M_n = 4600$, molecular weight distribution $M_w/M_n = 1.24$ in the methylated form). For the synthesis of the block copolymers, the crude poly(A-Pro-OH) was purified by precipitation from a MeOH solution into an excess solution of tetrahydrofuran (THF)/hexane (8/2 vol %), and the resulting product was dried under vacuum at room temperature; yield = 1.13 g, 94%. The molecular weight of poly(A-Pro-OH), $M_{n,NMR}$, was calculated by comparing the peak at 4.2–4.6 ppm corresponding to the methine proton adjacent to an carboxyl group (see Supporting Information, peak “f” in Figure S1) in A-Pro-OH repeating units with the peak at 7.8–7.9 ppm (peak “h”) corresponding to two protons of the end group. In the carboxylic acid form, $M_{n,NMR} = 4600$, and this was employed for the calculation of the feed ratio in the synthesis of the block copolymers, as mentioned below.

The dithiocarbamate-terminated poly(A-Pro-OH) (0.083 g, 0.018 mmol), AIBN (1.5 mg, 0.009 mmol), A-Pro-OMe (0.251 g, 1.36 mmol), and methanol (1.0 mL) were placed in a dry ampule. After the solution was degassed over three freeze–evacuate–thaw cycles, polymerization was conducted at 60 °C for 24 h (conversion determined by $^1$H NMR spectroscopy = 98%). The reaction mixture was purified by reprecipitation in diethyl ether and then isolated by filtration to yield the block copolymer poly(A-Pro-OH)-*b*-Poly(A-Pro-OMe) as a pale yellow powder. The methylation of the poly(A-Pro-OH) segment in the block copolymer was conducted by treating the carboxylic acid groups using trimethylsilyldiazomethane to afford poly(A-Pro-OMe)-*b*-poly(A-Pro-OMe), which was used for the SEC measurement. The methylated block copolymer has an $M_{n,GPC}$ value of 14 700, which corresponds to $M_n = 14\,300$ in the carboxylic acid form, and a polydispersity index ($M_w/M_n$) of 1.34.

The copolymer composition of poly(A-Pro-OH)-*b*-poly(A-Pro-OMe) was determined using $^1$H NMR spectroscopy and by comparing the peaks associated with the two comonomers. The peak at 3.5–4.0 ppm implies that both units comprise methylene protons ($NCH_2$) and the A-Pro-OMe unit consists of methyl protons; in contrast, the peaks at 4.0–4.6 ppm imply that both the units (NC*H*COO) comprise methine protons, as shown in Figure 2b. Thus, the comonomer composition can be calculated using eq 1 as follows:

$$\frac{2(x) + 5(1-x)}{x + 1(1-x)} = \frac{\text{integral at 3.5–4.0 ppm}}{\text{integral at 4.0–4.6 ppm}} \qquad (1)$$

Here $x$ is the fraction of A-Pro-OH, and $1 - x$ is the fraction of A-Pro-OMe in the block copolymer.

**Synthesis of Random Copolymers.** RAFT copolymerization was also performed with AIBN as an initiator in a degassed sealed tube. A representative example of the synthesis of the random copolymer is as follows: A-Pro-OH (0.077 g, 0.45 mmol), A-Pro-OMe (0.083 g, 0.45 mmol), benzyl 1-pyrrolecarbodithioate (4.46 mg, 0.018 mmol), AIBN (1.50 mg, 0.009 mmol), and methanol (0.64 mL) were placed in a dry ampule, and the solution was then degassed over three freeze–evacuate–thaw cycles. After the ampule was flame-sealed under vacuum, it was stirred at 60 °C for 24 h. The product was purified by precipitation into an excess amount of diethyl ether/ethyl acetate (7/3 vol %), and then it was dried under vacuum at room temperature to yield a copolymer as a pale yellow powder (yield = 99%). The methylated sample modified by the reaction of the carboxylic acid groups using trimethylsilyldiazomethane had an $M_{n,GPC}$ value of 7700, which corresponds to $M_n = 7100$ in the carboxylic acid form and a polydispersity index ($M_w/M_n$) of 1.30.

**Instrumentation.** The $^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra were recorded using a JEOL JNM-ECX400. The circular dichroism (CD) was measured using a JASCO J-720 spectropolarimeter, and the UV–vis spectra were recorded using a JASCO V-630BIO UV–vis spectrophotometer.

$M_n$ and $M_w/M_n$ were estimated by applying SEC at 40 °C using a Tosoh HPLC HLC-8220 system equipped with refractive index and ultraviolet detectors. The column set was as follows: four consecutive hydrophilic vinyl polymer-based gel columns [TSK-GELs (bead size, exclusion limited molecular weight): α-M (13 $\mu$m, $>1 \times 10^7$), α-4000 (10 $\mu$m, $4 \times 10^5$), α-3000 (7 $\mu$m, $9 \times 10^4$), α-2500 (7 $\mu$m, $5 \times 10^3$), 30 cm each] and a guard column [TSK guard column α, 4.0 cm]. The system

**Table 1. Synthesis of Block Copolymers by Polymerization of *N*-Acryloyl-L-proline Methyl Ester (A-Pro-OMe) Using 2,2′-Azobis(isobutyronitrile) (AIBN) and Poly(*N*-acryloyl-L-proline), Poly(A-Pro-OH), as a Macro-CTA in Methanol at 60 °C for 24 h**[a]

| | | | | | | A-Pro-OH/A-Pro-OMe composition | |
|---|---|---|---|---|---|---|---|
| run | [A-Pro-OMe]/[macro-CTA] | $M_n$[b] (theory) | $M_n$[c] (NMR) | $M_n$[d] (SEC) | $M_w/M_n$[d] (SEC) | calcd[e] | obsd[f] |
| 1 | 25 | 9 000 | 9 000 | 8 500 | 1.25 | 46:54 | 43:57 |
| 2 | 50 | 13 700 | 12 800 | 12 700 | 1.34 | 36:64 | 32:68 |
| 3 | 75 | 18 300 | 16 000 | 14 700 | 1.34 | 31:69 | 22:78 |
| 4 | 100 | 22 900 | 24 000 | 24 800 | 1.37 | 19:81 | 7:93 |

[a] Poly(A-Pro-OH) macro-CTA ($M_n$ = 4600, $M_w/M_n$ = 1.24), [macro-CTA]/[AIBN] = 2, monomer concentration = 0.25 g/mL. In all cases, conversion calculated by $^1$H NMR = 99%. [b] Theoretical molecular weight ($M_{n,theory}$) = (MW of A-Pro-OMe) × [A-Pro-OMe]/[macro-CTA] × conv + (MW of macro-CTA), A-Pro-OH = *N*-acryloyl-L-proline. [c] Evaluated by $M_n$ value of the poly(A-Pro-OH) macro-CTA and composition of the block copolymer determined by $^1$H NMR. [d] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N,N*-dimethylformamide (DMF, 0.01 M LiBr). [e] Calculated from the monomer conversion, $M_n$ value, of the macro-CTA, and the monomer composition in the feed. [f] Determined by $^1$H NMR in $CD_3OD$.

was operated at the flow rate of 1.0 mL/min using DMF containing 10 mM LiBr as the eluent. Polystyrene standards were employed for calibration.

The phase-separation temperatures of the aqueous solutions of the polymers (2.0 mg/mL) were measured by monitoring the transmittance of a 500 nm light beam through a quartz sample cell. The transmittance was recorded on a JASCO V-630BIO UV−vis spectrophotometer equipped with a temperature controller system (JASCO EHC-716 and EHC-717). The polymer solutions were filtered before the measurement using Millipore Teflon filters with a pore size of 0.2 μm. The temperature was increased at a rate of 1.0 °C/min with heating scans between 10 and 70 °C. Elemental analysis was carried out on a Perkin-Elmer 2400II CHNS/O analyzer. First, the polymer solutions were filtered using Millipore Teflon filters with a pore size of 0.2 μm into a dust-free cylindrical cuvette. Subsequently, dynamic light scattering (DLS) was performed at room temperature using an Otsuka Electronics ELS-800 spectrometer with a He−Ne laser ($\lambda_0$ = 632.8 nm) at a scattering angle of 90°.

## Results and Discussion

**Synthesis of Block Copolymers.** In a previous communication, we reported that the polymerization of A-Pro-OMe with benzyl 1-pyrrolecarbodithioate at 60 °C afforded the nearly monodispersed poly(A-Pro-OMe) with controlled molecular weights.[41] Furthermore, good control of the polymerization of A-Pro-OH was attained by RAFT polymerization with the same dithiocarbamate-type chain transfer agent (CTA) under similar conditions.[48] For the synthesis of well-defined block copolymers using the RAFT process, the order of blocking is crucial. In this case, the first dithioester-terminated polymer (S═C(Z)S−A; A block corresponds to the first polymer, whereas Z is the stabilizing group) should have a high transfer constant in the subsequent polymerization of second monomers to yield B block.[61,62] In order to obtain a block copolymer with low polydispersity, the rapid conversion of macro-CTA to a block copolymer is also required; in this manner, all the second blocks can be initiated at approximately the same time.[63,64] Since there is no significant difference in the resonance effect of both propagating radicals in this system, it is reasonable to expect that a higher steric hindrance of the macro-CTA leads to a higher fragmentation ratio of the intermediate to the first propagating polymer radical under the selected reaction conditions. Therefore, for the controlled synthesis of the dual-stimuli-responsive block copolymers derived from L-proline derivatives, we conducted RAFT polymerization of A-Pro-OMe using the poly(A-Pro-OH) macro-CTA having the dithiocarbamate end group, as shown in Scheme 1.

The dithiocarbamate-terminated poly(A-Pro-OH) was first prepared by the RAFT polymerization of A-Pro-OH with benzyl 1-pyrrolecarbodithioate. The synthesis of the block copolymer was conducted using the poly(A-Pro-OH) macro-CTA ($M_n$ = 4600, $M_w/M_n$ = 1.24) in methanol at 60 °C for 24 h, while maintaining the macro-CTA to initiator ratio at a constant value of $[\text{macro-CTA}]_0/[\text{AIBN}]_0$ = 2/1. The monomer to macro-CTA ratio ($[\text{A-Pro-OMe}]_0/[\text{macro-CTA}]_0$) was varied from 25 to 100 in order to control the comonomer content and molecular weight. Under these conditions, the conversions determined by $^1$H NMR were almost quantitative (>99%) in all the cases. As shown in Table 1, the composition of each segment and the molecular weights of the resulting block copolymers could be adjusted by the $[\text{A-Pro-OMe}]_0/[\text{macro-CTA}]_0$ ratio in the feed. Figure 1 shows the SEC chromatograms of the starting macro-CTA and growth polymers, which were obtained by the polymerization, followed by the methylation of the carboxylic acid groups. A shift in the SEC trace toward higher molecular ranges can be observed as the [A-Pro-OMe]/[macro-CTA] ratio increases. The molecular weight distributions remain narrow ($M_w/M_n$ = 1.25−1.37), and the SEC traces are unimodal with no evidence of high molecular weight species and homopolymer impurity. In the $^1$H NMR spectrum of the block copolymer measured in $CD_3OD$, the peaks corresponding to both components are clearly detected (Figure 2). The compositions of the copolymers determined by $^1$H NMR are in reasonable agreement with the calculated values from the conversion and feed ratios of both monomers, irrespective of the comonomer ratio in the feed. It was found that the dithiocarbamate-terminated poly (A-Pro-OH) macro-CTA can be successfully employed for the synthesis of the block copolymer with a relatively narrow polydispersity, controlled molecular weights, and predetermined composition. In other words, these results suggest that

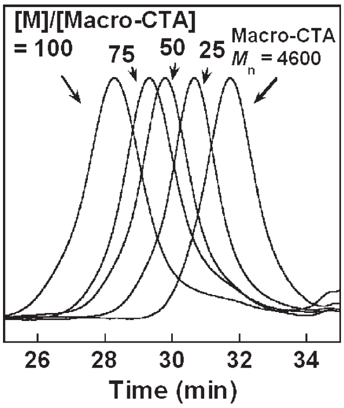


**Figure 1.** SEC traces of methylated block copolymers obtained by the polymerization of A-Pro-OMe using the dithiocarbamate-terminated poly(A-Pro-OH) at different [A-Pro-OMe]/[macro-CTA] ratios, followed by methylation. See Table 1 for detailed polymerization conditions.

the fragmentation from the intermediate radical to the poly(A-Pro-OH) radical combined with an efficient reinitiation is sufficiently effective; this results in the rapid conversion of the macro-CTA to the block copolymer under the conditions employed in this study.

For comparison, we synthesized proline-based random copolymers using the RAFT copolymerization of A-Pro-OMe with A-Pro-OH, by which an appropriate hydrophilic/hydrophobic balance can be achieved. The copolymerization via the RAFT process was conducted using benzyl 1-pyrrolecarbodithioate as the CTA in methanol at 60 °C at different A-Pro-OMe:A-Pro-OH molar ratios in the feed at ([A-Pro-OMe] + [A-Pro−OH])/[CTA] = 50 and [CTA]/[AIBN] = 2. The results are summarized in Table 2. Depending on the comonomer ratio (A-Pro-OMe:A-Pro-OH = 75:25, 50:50, and 25:75) in the feed, copolymers with $M_n$ between 7400 and 7900 and the A-Pro-OH content between 26% and 73% were obtained quantitatively (yield = 99%). The polydispersity indices ($M_w/M_n$) for all the samples ranged between 1.30 and 1.31, and the SEC traces were unimodal with no evidence of high molecular weight species (see Supporting Information). The molecular weight distribution and comonomer composition of the resulting random copolymers are comparable to those of block copolymers.

**Thermal Phase Transition in Aqueous Solutions.** In this study, poly(A-Pro-OMe) was selected as a thermo-responsive segment, whereas poly(A-Pro-OH) was employed as a pH-responsive segment. Poly(A-Pro-OMe) is soluble in most organic solvents such as dichloromethane, chloroform, acetone, ethyl acetate, THF, dioxane, methanol, DMF, and DMSO, while it is insoluble in diethyl ether and hexane. Poly(A-Pro-OMe), which exhibits a soluble-insoluble transition at 18 °C, was observed to have a characteristic LCST-type thermo-responsive property in neutral water (pH = 7). In contrast, poly(A-Pro-OH) was soluble in basic water (pH = 10), methanol, DMF, and dioxane and insoluble in neutral water (pH = 7), acidic water (pH = 1), chloroform, acetone, ethyl acetate, THF, diethyl ether, and hexane. Hence, the block copolymers comprising poly(A-Pro-OMe) and poly(A-Pro-OH) were soluble in methanol and DMF, regardless of the comonomer composition.

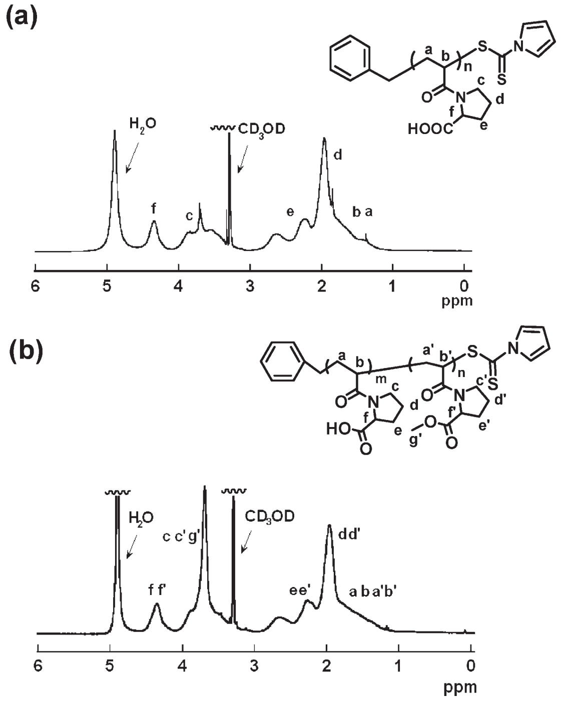


**Figure 2.** $^1$H NMR spectra of (a) poly(A-Pro-OH) and (b) poly(A-Pro-OH)-*b*-poly(A-Pro-OMe) in $CD_3OD$.

We initially evaluated thermally induced phase separation behaviors of poly(A-Pro-OH)$_{22}$-*b*-poly(A-Pro-OMe)$_{78}$ in water at different pH values, as monitored by UV (500 nm), in which the heating rate was fixed at 1.0 °C/min. The block copolymer was insoluble in water at pH = 7 and 8 at room temperature, while LCST-type phase transition was observed at pH > 9. These results are consistent with the observed solubility of each segment in the block copolymer, that is, the case in which both poly(A-Pro-OH) and poly(A-Pro-OMe) were insoluble in neutral water (pH = 7) at room temperature. In contrast, the weak polyelectrolyte, poly(A-Pro-OH), was soluble in water at pH > 9, which may be due to the partial ionization of carboxylic acid moiety. As can be seen in Figure 3a, the block copolymer was soluble in water at pH = 9 at low temperature, which undergoes a clear phase transition upon heating. The transmittance decreased gradually from 20 to 40 °C, indicating a relatively slow phase separation. At pH = 10, a sharp transition was observed from 35 to 40 °C, and a different transition behavior was detected at pH = 11. The phase transition temperature was varied from around 25 °C at pH = 9 to 55 °C at pH = 11, and the LCST increased linearly with increasing pH value (Figure 3b), which can be attributed to the higher degree of ionization of the poly(A-Pro-OH) segment. These results suggest that the pH value in aqueous solutions affects both phase separation temperature and behavior. It is also demonstrated that the proline-based block copolymer having a well-defined structure shows characteristic pH- and temperature-responsive properties, in which the transition temperature can be manipulated in the range of 25−55 °C by the pH values.

The thermally induced phase separation behavior of the block copolymer, poly(A-Pro-OH)$_{22}$-*b*-poly(A-Pro-OMe)$_{78}$, was compared with that of the corresponding random copolymer, poly(A-Pro-OH-*co*-A-Pro-OMe), at pH = 10 and 11. As shown in Figure S4 (see Supporting Information), the random copolymer having A-Pro-OH content = 26% was insoluble at pH = 10 and soluble at pH = 11 in water, independent of the temperature; this indicates no LCST-type phase separation behavior. This may be due to the fact that

**Table 2. Random Copolymerization of *N*-Acryloyl-L-proline (A-Pro-OH) and *N*-Acryloyl-L-proline Methyl Ester (A-Pro-OMe) in the Presence of Chain Transfer Agent (CTA) at 60 °C for 24 h**[a]

| run | feed ratio (A-Pro-OH:A-Pro-OMe) | $M_n$[b] (theory) | $M_n$[c] (SEC) | $M_w/M_n$[c] (SEC) | m:n[d] (NMR) |
|---|---|---|---|---|---|
| 1 | 75:25 | 8900 | 7900 | 1.31 | 73:27 |
| 2 | 50:50 | 8700 | 7700 | 1.30 | 52:48 |
| 3 | 25:75 | 8600 | 7400 | 1.31 | 26:74 |

[a] ($[A\text{-}Pro\text{-}OH]_0+[A\text{-}Pro\text{-}OMe]_0)/[CTA]_0=50$, $[CTA]_0/[AIBN]_0=2$, monomer concentration = 0.25 g/mL, where AIBN = 2,2′-azobis(isobutyronitrile), A-Pro-OH = *N*-acryloyl-L-proline, A-Pro-OMe = *N*-acryloyl-L-proline methyl ester. Isolated yield after precipitation with diethyl ether:acetone (7:3 vol %) = 99% in all cases. [b] Theoretical molecular weight ($M_{n,\text{theory}}$) = (MW of A-Pro-OH$_m$ + A-Pro-OMe$_n$) × [A-Pro-OH + A-Pro-OMe]/[macro-CTA] × yield + (MW of CTA). [c] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr). [d] Comonomer composition (m = A-Pro-OH, n = A-Pro-OMe) determined by $^1$H NMR in $CD_3OD$.

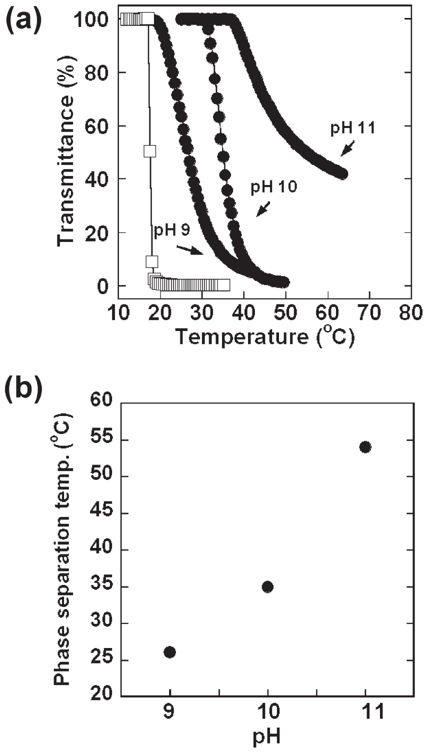


**Figure 3.** (a) Temperature dependence of the transmittance at 500 nm of aqueous solutions (2.0 mg/mL) of (●) poly(A-Pro-OH)$_{22}$-*b*-poly(A-Pro-OMe)$_{78}$ ($M_n$ = 14 700, $M_w/M_n$ = 1.34) at different pH values and (□) poly(A-Pro-OMe) at pH 7. (b) Relationship between the phase separation temperature and pH value of the aqueous solution of the block copolymer.

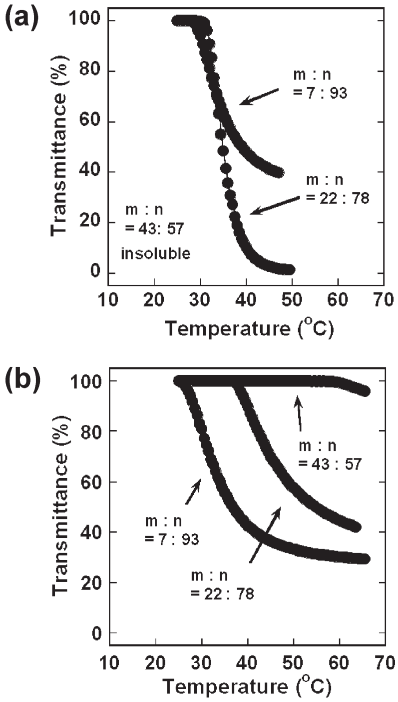


**Figure 4.** (a) Temperature dependence of the transmittance at 500 nm of the aqueous solutions (2.0 mg/mL) of poly(A-Pro-OH)$_m$-*b*-poly(A-Pro-OMe)$_n$ having different compositions at (a) pH = 10 and (b) pH = 11.

the relatively homogeneous incorporation of A-Pro-OH component in the random copolymer prevents the formation of poly(A-Pro-OMe) segments with sufficient chain lengths, which results in the disappearance of the thermo-responsive property.

Figure 4 shows the dependence of the solution turbidity of the block copolymers having different comonomer contents on the temperature. The mole fractions of A-Pro-OH, *m*, and the pH value were obtained to determine the temperature of the phase separation. In the case of the block copolymer having lower A-Pro-OH content (*m* = 7) at pH = 10, the transmittance decreased gradually, varying from 100% at 30 °C to 40% at 50 °C, as shown in Figure 4a. The increase in the A-Pro-OH content (*m* = 22) in the block copolymer led to a clear phase transition from 100% at 30 °C to 0% at 50 °C. Furthermore, the block copolymer having higher A-Pro-OH content (*m* = 43) was not dissolved directly in water at pH = 10. In contrast, a different behavior was observed at pH = 11 (Figure 4b); here, the block copolymer having higher A-Pro-OH content (*m* = 43) was soluble in basic water to afford a transparent solution, independent of the temperature. These results also suggest that the block copolymers having suitable comonomer compositions (*m* = 7 and 22) show characteristic dual-stimuli-responsive behaviors, while the introduction of more A-Pro-OH segments results in the loss of temperature-responsive property to yield simple pH-responsive block copolymers.

For the block copolymers with relatively lower A-Pro-OH contents at pH = 11, the transparent solution observed at 20 °C was changed into turbid solutions (transparency = 50% at *m* = 22 and 30% at *m* = 7) at 50 °C. The result observed at pH = 11 is consistent with the general tendency that the LCST depends on the distribution of hydrophilic and hydrophobic groups within the polymers and that the transition temperature increases with increasing hydrophilicity. However, at pH = 10, an LCST shift to a lower temperature was observed with the incorporation of more A-Pro-OH segments in the block copolymer. The opposite tendency is the reason for the formation of the hydrogen bonds between the two components. Since a disubstituted amide is known to be a powerful hydrogen acceptor,[65,66] it is reasonable to suggest efficient hydrogen bonding between the amide moiety in both the proline-based units and carboxylic acid moiety in A-Pro-OH. At pH = 10, the carboxylic acid in A-Pro-OH is partially ionized, and the hydrogen bonds between the amide moiety and carboxylic acid are predominant. The formation of the hydrogen bonds protects A-Pro-OMe groups from exposure to water and results in a hydrophobic contribution to the LCST. A similar effect of the hydrogen bonding on pH/temperature-induced phase separation was observed in other random copolymers involving poly((*N*,*N*-dimethylamino)ethyl methacrylate-*co*-ethylacrylamide)[66] and (*N*,*N*-dimethylamino)ethyl methacrylate-*co*-acrylamide).[67] At pH = 11, an almost fully charged A-Pro-OH chain leads to the breaking of the hydrogen bonding, and a A-Pro-OH component acts as a simple hydrophilic segment, which leads to an increase in LCST.

Since poly(A-Pro-OH) is a weak polyelectrolyte, the degree of ionization is changed by the ionic strength of the aqueous solution, and the addition of salt will screen the anionic charges. Salt is also known to affect the phase behavior of the aqueous solution of a representative thermo-responsive polymer, poly(*N*-isopropylacrylamide), because they disrupt the hydration structure surrounding the polymer chains.[68,69] The effect of salt concentration on the thermo-responsive properties of the proline-based block copolymer was investigated at pH = 10 and 11. A block copolymer having an intermediate A-Pro-OH content (*m* = 37) was employed, and the measurement was conducted

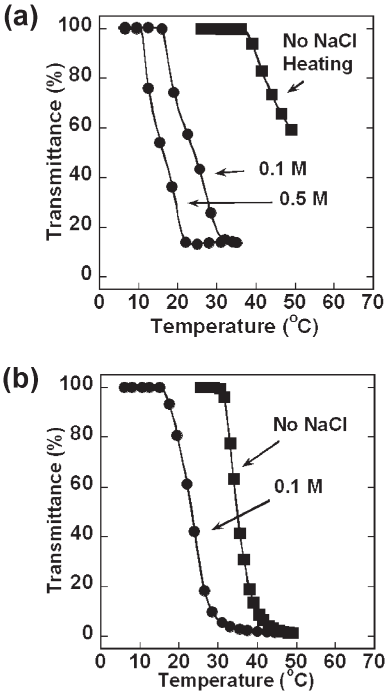


**Figure 5.** Temperature dependence of the transmittance at 500 nm of aqueous solutions (2.0 mg/mL) of poly(A-Pro-OH)$_{37}$-*b*-poly(A-Pro-OMe)$_{63}$ at (a) pH = 11 and (b) pH = 10 with different salt concentrations; [NaCl] = 0.1 and 0.5 M.

in an aqueous solution (concentration = 2 mg/mL) at different NaCl concentrations (0.1−1.0 M). At room temperature, the block copolymer was soluble without salt at pH = 11, and the transmittance decreased gradually from 100% at 37 °C to 60% at 50 °C. As shown in Figure 5a, the LCST-type phase separation is observed at [NaCl] = 0.1 and 0.5 M, and the decrease in the transition temperature is observed with increasing NaCl concentration. In both cases, this phase separation was reversible on heating and cooling without hysteresis (see Supporting Information, Figure S5). At higher salt concentrations ([NaCl] = 1.0 M), the block copolymer was insoluble at pH = 11, regardless of the temperature. At pH = 10, the addition of NaCl into the aqueous solution led to a decrease in the transition temperature at [NaCl] = 0.1 M (Figure 5b), whereas the block copolymer was insoluble even at [NaCl] = 0.5 M. In both cases, the higher salt concentration resulted in the loss of water solubility at room temperature as well as loss of thermo-responsive property. These results indicate that the characteristic pH- and thermo-responsive properties of the proline-based block copolymer can be manipulated by varying the salt concentration.

**Solution and Chiroptical Properties of Block Copolymers.** The solution properties of a representative block copolymer were characterized using DLS in basic water (pH = 10), which is a good solvent only for poly(A-Pro-OH). As shown in Figure 6, the block copolymer shows a monomodal hydrodynamic diameter distribution in the selective solvent at 20 °C. The relatively narrow hydrodynamic diameter distribution most probably indicates a spherical morphology of the micelles consisting of a relatively hydrophobic core of poly(A-Pro-OMe) and a hydrophilic shell of poly(A-Pro-OH). Note that the formation of the hydrogen bonds between the amide moiety in both the proline-based units and carboxylic acid moiety in A-Pro-OH may protect the A-Pro-OMe groups from exposure to water, even if poly(A-Pro-OMe) exhibits the transition temperature at around 20 °C. The increase in the solution temperature leads to an increase in the average hydrodynamic diameter; $D_h$ = 127 nm at 20 °C and 257 nm at 60 °C. Figure 7 shows the temperature dependence of $D_h$ of the aqueous solution (pH = 10, concentration = 2 mg/mL) of poly(A-Pro-OH)$_{22}$-*b*-poly(A-Pro-OMe)$_{78}$. This behavior may be a result of the formation of stable core−shell micelles with a hydrophobic core formed by the aggregation of dehydrated A-Pro-OMe blocks and a water-soluble shell formed by ionized A-Pro-OH blocks above the phase separation temperature. The dehydration of poly(A-Pro-OMe) segment appears to occur progressively, and the micelle size increases as the dehydration proceeds.

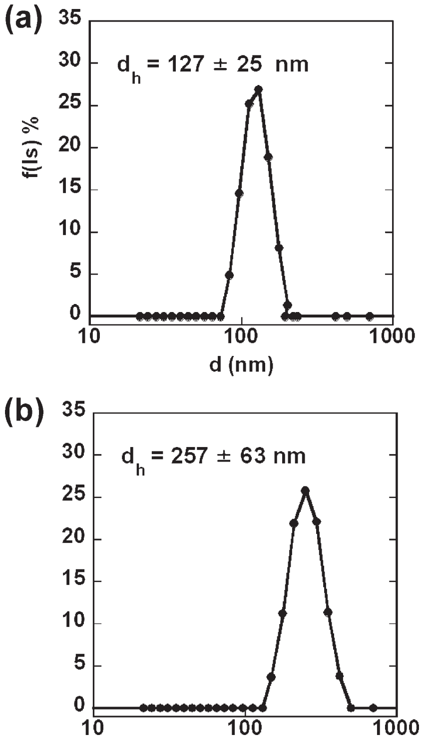


**Figure 6.** Hydrodynamic diameter distributions *f*($D_h$) of the micelles obtained from poly(A-Pro-OH)$_{22}$-*b*-poly(A-Pro-OMe)$_{78}$ in basic water (pH = 10, polymer concentration = 2 mg/mL) at (a) 20 °C and (b) 60 °C.

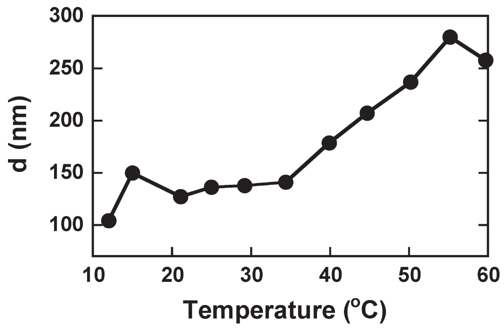


**Figure 7.** Temperature dependence of hydrodynamic diameter ($D_h$) of aqueous solution (pH = 10, concentration = 2 mg/mL) of poly(A-Pro-OH)$_{22}$-*b*-poly(A-Pro-OMe)$_{78}$.

In recent times, there has been increasing focus on the development of novel amino acid-based block copolymers comprising chiral−chiral components,[38,39,46,70] in addition to conventional chiral−achiral type block copolymers. In terms of chirality, the poly(A-Pro-OH)$_m$-*b*-poly(A-Pro-OMe)$_n$ prepared in this study can be classified into a chiral−chiral (L−L) type block copolymer. The chiroptical

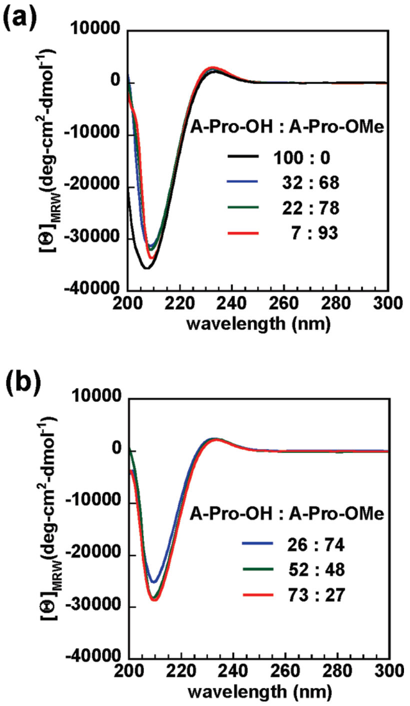


**Figure 8.** CD spectra ($c$ = 0.03 mg/mL) of (a) poly(A-Pro-OH) and the block copolymers having different compositions, poly(A-Pro-OH)$_m$-*b*-poly(A-Pro-OMe)$_n$, and (b) the corresponding random copolymers, poly(A-Pro-OH$_m$-*co*-A-Pro-OMe$_n$), in basic water (pH = 12).

behavior of the block copolymer, which is related to the conformation of polymers in solution, was investigated by the CD measurement. Figure 8 depicts the CD spectra of the block copolymers having different compositions, poly(A-Pro-OH)$_m$-*b*-poly(A-Pro-OMe)$_n$, and those of the corresponding random copolymers in basic water (pH=12, polymer concentration = 0.03 mg/mL). Under these conditions, all the block and random copolymers were soluble in basic water at room temperature, independent of the composition. The block copolymers show a strong negative signal at 210 nm, which is very probably due to the n → π* transition of the carboxyl chromophore.[71] The peak intensities of the negative peaks of the block copolymers (−30 000−35 000 deg cm$^2$ dmol$^{-1}$) are comparable to those of the homopolymer poly(A-Pro-OH). As shown in Figure 8a,b, there is a slight decrease in the peak intensities of the random copolymers (−25 000−28 000 deg cm$^2$ dmol$^{-1}$, Figure 8b), which suggests the existence of a specific conformation of the block copolymer in basic water. In both cases, a positive signal is visible at around 230 nm, which is attributed to the $\pi_1 \rightarrow \pi^*$ transition of the amide chromophore.[71]

## Conclusion

We have demonstrated the controlled synthesis of novel dual-stimuli-responsive block copolymers composed of the thermo-responsive poly(A-Pro-OMe) and pH-responsive poly(A-Pro-OH) by RAFT polymerization of two proline-based monomers. Precise control of the molecular weight, polydispersity, and composition of the novel block copolymers could be achieved by the RAFT polymerization of A-Pro-OMe using poly(A-Pro-OH) as a hydrophilic macro-CTA under suitable conditions. This work presents the feasibility of manipulating characteristic thermo-responsive properties by using the pH value along with the salt concentration and composition of two chiral monomers. The formation of the micelles consisting of a hydrophobic core of poly(A-Pro-OMe) and a hydrophilic shell of poly(A-Pro-OH) was observed in a basic aqueous solution (pH = 10). The assembled structure induced by the selective solvent affected the characteristic thermo-responsive property, whereas there was no significant effect on the chiroptical property.

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**Supporting Information Available:** Figures showing $^1$H NMR spectra of the poly(A-Pro-OH) used as a macro-CTA and a random copolymer, SEC trace of a methylated random copolymer, temperature dependence of the transmittance of a random copolymer at different pH values, and heating−cooling cycle of the block copolymer at different salt concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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