RAFT Polymerization of Acrylamides Containing Proline and Hydroxyproline Moiety: Controlled Synthesis of Water-Soluble and Thermoresponsive Polymers

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ABSTRACT: Two acrylamides containing proline and hydroxyproline moiety, *N*-acryloyl-L-proline (A-Pro-OH) and *N*-acryloyl-4-*trans*-L-proline (A-Hyp-OH), were polymerized by reversible addition-fragmentation chain transfer (RAFT) process to afford well-defined amino acid based polymers. Two chain transfer agents (CTAs), benzyl dithiobenzoate (CTA 1) and benzyl 1-pyrrolecarbodithioate (CTA 2), were compared for the direct polymerization of these monomers without protecting chemistry. With 2,2′-azobis(isobutyronitrile) as an initiator, the dithiocarbamate-type RAFT agent (CTA 2) was efficient for the controlled synthesis of poly(A-Pro-OH)s, which can be regarded as a weak polyelectrolyte. Controlled character of the polymerization of A-Hyp-OH, which has a carboxylic acid and a hydroxyl group in the monomer unit, in the presence of CTA 1 was confirmed by the formation of narrow polydispersity products and the linear relationship between the molecular weight and conversion. Water-soluble poly(A-Hyp-OH)s with number-average molecular weights between 8.2×10^3 and 2.21×10^4 and relatively low polydispersities were obtained, depending on the monomer/CTA ratio. Their methylated samples, poly(A-Pro-OMe) and poly(A-Hyp-OMe), and random copolymers showed characteristic thermal phase transitions in aqueous solutions.

Introduction

Proline and hydroxyproline are of biological relevance because they are the major constituents of collagen, which is the most abundant protein in animals and the major fibrous element of skin, bone, tendon, cartilage, and teeth.[1–3] Collagen consists of three individual peptide strands that fold into a triple helix, and each strand contains many repeats of the sequence, X-Y-Gly, where usually X = L-proline and Y = 4-(*R*)-hydroxyproline.[4–6] During recent years, there has been an increasing attention paid to structures and conformational stability of the triple helix of various tripeptide collagen models.[4–6] The 4-hydroxyproline residues greatly enhance the conformation stability of the collagen triple helix, and the hyperstability arises from stereoelectronic effects that preorganize the main-chain dihedral angles in the conformation found in the triple helix.[3,7,8] A variety of polymers based on hydroxyproline have been reported, including polyester,[9–11] polyester copolymer,[12–14] polyamide,[15,16] and polyacrylate[17] derivatives. L-Proline is also responsible for various functions and ordered structures, such as a turn inducer in natural peptides and proteins[18,19] and the helix structure of oligoprolines.[20,21] The attractive features of L-proline have prompted a number of studies of derivatives,[22,23] polymeric materials,[24] and their biorelated phenomena and applications.[25,26]

Amino acid−based polymers have been extensively investigated for various biorelated applications, which can be divided into two groups: polymers containing amino acid moieties in the main chain or side chain.[27] A variety of polymers having amino acid moieties in the side chain has been synthesized by conventional free radical polymerization of vinyl monomers carrying amino acid residues. Recently, much attention has been paid to develop precise synthetic methods to control molecular weight, polydispersity, topology, composition, sequence, and functions, in order to utilize noncovalent bonds between amino acid residues for the self-organization behavior of synthetic polymers and their unique properties and functions.[28] Atom transfer radical polymerization,[29–31] nitroxide-mediated radical polymerization,[32] and reversible addition-fragmentation chain transfer (RAFT) polymerization[33–46] are powerful tools for the synthesis of well-defined functional polymers. These controlled radical polymerization methods were recently employed for the synthesis of a variety of peptide−polymer hybrids having well-defined structures and characteristic properties.[47–55] As a part of our continuous effort to develop amino acid−based highly ordered architectures, we reported controlled radical polymerization of acrylamides having various amino acid moieties in the side chains, *N*-acryloyl-L-phenylalanine methyl ester,[56] *N*-acryloyl-L-phenylalanine,[57] and *N*-acryloyl-L-proline methyl ester (A-Pro-OMe),[58–60] via the RAFT process. We also focused on thermoresponsive random and block copolymers involving A-Pro-OMe units, which exhibited a characteristic LCST (lower critical solution temperature) in aqueous solution.[58,60] Aqueous RAFT polymerization of a similar *N*-acryloyl derivative was also employed for the syntheses of well-defined block copolymers containing amino acid based segments.[53,54]

In this contribution, we selected proline and hydroxyproline as starting materials for the preparation of functional monomers due to their biological relevance, commercial availability, chirality, and amphiphilic nature. In the first part, we present direct RAFT polymerization of *N*-acryloyl-L-proline (A-Pro-OH), in which the carboxylic acid moiety is intact without a protecting group, as shown in Scheme 1. Controlled polymerization of amino acid−based monomers with carboxylic acid moiety is promising for producing tailored functional polymers for various applications, because of the abilities of carboxylic acid groups to interact with various metal ions, proton-accepting polymers, and cationic polyelectrolytes[61] Carboxylic acid

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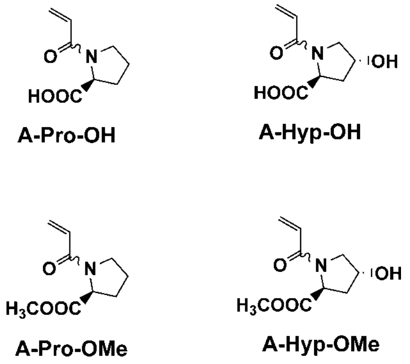
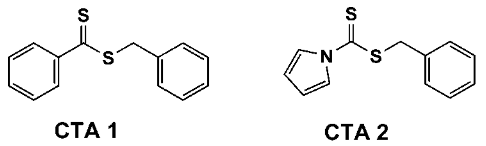
Scheme 1



Scheme 2



containing polymers can be also regarded as weak polyelectrolytes, in which the degree of ionization is governed by the pH and ionic strength of aqueous solution. Such tuning capabilities are of great interest as a component of intelligent materials. Then, we describe the conditions best suited toward the controlled polymerization of *N*-acryloyl-4-*trans*-hydroxy-L-proline (A-Hyp-OH), which has a carboxylic acid and a hydroxyl group in the monomer unit, without protecting chemistry. Poly(A-Hyp-OH) can be also regarded as a strong hydrophilic polymer and a precursor of thermoresponsive poly(A-Hyp-OMe). Poly(A-Hyp-OH) can show specific interactions, such as hydrogen-bonding, acid−base interactions, and oppositely charged ionic interactions, which can help to prepare novel self-organized materials. Furthermore, the carboxylic acids and hydroxyl groups in poly(A-Hyp-OH) can be used as sites to bind biorelated materials, such as proteins. Finally, the thermal phase transitions of aqueous solutions of poly(A-Pro-OMe), poly(A-Hyp-OMe), and their random copolymers prepared by methylation reaction of the carboxylic acid groups were studied as a part of our continuous efforts to the design and synthesis of intelligent amino acid−based polymers with characteristic stimuli-responsive properties.

In this study, we selected two different chain transfer agents (CTAs), namely benzyl dithiobenzoate (CTA 1) and benzyl 1-pyrrolecarbodithioate (CTA 2), as shown in Scheme 2. The right selection of CTA, as the choice of R and Z groups depends on the monomer, acts frequently as a critical role in determining the overall success of a RAFT polymerization with respect to control over the molecular weight and molecular weight distribution.[35,37–39] We previously demonstrated that controlled radical polymerization of monosubstituted acrylamides having phenylalanine moieties, *N*-acryloyl-L-phenylalanine methyl ester[56] and *N*-acryloyl-L-phenylalanine,[57] was attained by RAFT process using CTA 2. An important monosubstituted acrylamide, *N*-isopropylacrylamide, was polymerized successfully via RAFT using CTA 2.[62] Whereas CTA 1 was efficient to achieve controlled polymerization of A-Pro-OMe, which afforded well-defined poly(A-Pro-OMe), and their random and block copolymers.[58–60] CTA 1 was employed as a RAFT agent for well-controlled polymerization of *N,N*-dimethylacrylamide[63] and *N*-isopropylacrylamide.[64] CTA 1 and CTA 2 have the same R group, which yields a benzyl radical species upon fragmentation. As the Z group influences strongly the stability of the thiocarbonylthio intermediate radical, strong stabilizing groups will favor the formation of the intermediate radical, and therefore enhance the reactivity of the S=C bond toward radical addition. However, the stability of the intermediate needs to be finely tuned to favor the fragmentation, which will release the reinitiating group (R). The CSIRO group recently reported the general guideline for selection of RAFT agents for particular monomers.[35,39] Successful implementation of the RAFT process also requires careful selections of the CTA/initiator ratio and reaction conditions involving solvent and temperature, depending upon the monomer.

Experimental Section

Materials. 2,2′-Azobis(isobutyronitrile) (AIBN, Kanto Chemical, 97%) was purified by recrystallization from methanol. L-Proline (Kanto Chemical, 99%), 4-*trans*-hydroxy-L-proline (Aldrich, 99%), methanol (dehydrated MeOH, Kanto Chemical, 99.8%), ethanol (dehydrated EtOH, Wako Pure Chemical, 99.5%), and *N,N*-dimethylformamide (dehydrated DMF, Kanto Chemical, 99.5%) were used as received. The methylation agent, trimethylsilyldiazomethane (2 M solution in diethyl ether), was purchased from Aldrich and used as received. Other materials were used without further purification.

Synthesis of Chain Transfer Agents (CTAs). The syntheses of benzyl dithiobenzoate (CTA 1)[58,65] and benzyl 1-pyrrolecarbodithioate (CTA 2)[66,67] were conducted according to the procedures reported previously. The CTA 2 was finally purified by column chromatography on silica with *n*-hexane as the eluent to afford the corresponding product as a yellow oil. CTA 1 was purified by vacuum distillation using a glass tube oven (Shibata GTO-250RS) to give a red oil. The elemental analyses of the CTAs gave calculated purities of >99% for CTA 2 and 98% for CTA 1.

***N*-Acryloyl-L-Proline (A-Pro-OH).** The monomer was prepared by the reaction of acryloyl chloride with L-proline according to a method reported previously with a slight modification.[68] Acryloyl chloride (2.16 g, 24.0 mmol) was added dropwise to an aqueous solution of 2.76 g (24.0 mmol) of L-proline in 50 mL of 2 wt % NaOH while the reaction mixture was kept at 0 °C by external ice-bath cooling. After the addition, the mixture was stirred for 1 h with the temperature raising to room temperature. The mixture was acidified to pH = 3 with 6 N HCl, and then the stirring was continued at room temperature for additional 1 h. The resulting mixture was extracted three times with chloroform (100 mL × 3) and the solution was dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the resulting solid was purified by recrystallization from ethyl acetate to give 1.36 g (8.05 mmol) of A-Pro-OH as a white solid (yield = 34%). $[\alpha]_D^{25} = -117.5°$, $c = 0.1$ g/dL, MeOH, and mp = 116 °C (lit.[68] mp = 118−120 °C). ^1H NMR (CD_3OD): δ 1.92−2.10 (m, 2H, $>NCH_2-CH_2-$), 2.21−2.31 (m, 2H, $-CH_2-CHCOOH$), 3.58−3.80 (m, 2H, $>NCH_2-$), 4.47−4.69 (m, 1H, $>CHCOOH$), 5.68−5.78 (m, 1H, $-CH=CH_2$), 6.22−6.67 (m, 2H, $-CH=CH_2$) ppm. ^{13}C NMR (CD_3OD): δ 23.8 ($>NCH_2-CH_2-$), 25.6 ($-CH_2-CHCOOH$), 30.2−32.2 ($>NCH_2-$), 60.2 ($>CHCOOH$), 128.9 ($-CH=CH_2$), 129.9 ($-CH=CH_2$), 167.1−167.4 ($-CONH-$), 175.0−175.4 ($-COOH$) ppm. Anal. Calcd for $C_8H_{11}NO_3$: C, 56.80; H, 6.55; N, 8.28. Found: C, 56.65; H, 6.57; N, 8.22. As shown in Figure S1 (see Supporting Information), the ^1H and ^{13}C NMR spectra of the monomer (A-Pro-OH) suggested the existence of the s-*cis* and s-*trans* forms, which is similar to the case of A-Pro-OMe.[24] The isomerization of the proline amide moiety between s-*cis* and s-*trans* forms has been widely studied in the fields of protein and peptide chemistry.

***N*-Acryloyl-4-Trans-Hydroxy-L-Proline (A-Hyp-OH).** The synthesis of A-Hyp-OH was also conducted similar to the preparation of A-Pro-OH by replacing L-proline with 4-*trans*-hydroxy-L-proline. Acryloyl chloride (2.16 g, 24.0 mmol) was added dropwise to an aqueous solution of 3.14 g (24.0 mmol) of 4-*trans*-hydroxy-L-proline in 25 mL of 5 wt % NaOH while the reaction mixture was kept at 0 °C by external ice-bath cooling. After the mixture was stirred at room temperature for 1 h, the mixture was acidified to pH = 2 with 6 N HCl. The stirring was continued at room temperature for additional 1 h. After the solution was extracted 10 times with ethyl acetate (30 mL × 10), and the washings were dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the resulting solid was purified by recrystallization from a mixture of ethyl acetate and acetone (8/2-vol ratio) to give 1.02 g (5.52 mmol) of A-Hyp-OH (yield = 20%) as a white solid. $[\alpha]_D^{25} = -106.9°$, $c = 0.1$ g/dL, MeOH. ^{1}H NMR (D_2O): δ 2.08−2.33 (m, 2H, >CH−CH_2−), 3.66−3.81 (m, 2H, >N−CH_2−), 4.44−4.50 (m, 2H, >C*H*OH, >NC*H*COOH), 5.77−5.78 (m, 1H, −C*H*=CH$_2$), 6.22−6.50 (m, 2H, −CH=CH_2) ppm. ^{13}C NMR (D_2O): δ 20.8−23.1 (−*C*H$_2$−CHCOOH), 47.3−47.6 (>N*C*H$_2$−), 47.8 (>*C*HCOOH), 60.0−62.2 (>*C*HOH), 134.4 (−CH=*C*H$_2$), 137.3 (−*C*H=CH$_2$), 184.3−185.5 (−*C*ONH−), 194.6−194.8 (−*C*OOH) ppm. Anal. Calcd for $C_8H_{11}NO_4$: C, 51.89; H, 5.99; N, 7.56. Found: C, 51.74; H, 5.76; N, 7.52. The ^{1}H and ^{13}C NMR spectra of the monomer are shown in Figure S3 (see Supporting Information), suggesting the existence of s-*cis* and s-*trans* forms.

General Polymerization Procedure. All polymerizations were carried out with AIBN as an initiator in a degassed sealed tube. A representative example is as follows: A-Pro-OH (0.386 g, 2.28 mmol), CTA 2 (10.65 mg, 0.045 mmol), AIBN (1.5 mg, 0.009 mmol), and dehydrated methanol (1.54 mL) were placed in a dry glass ampule equipped with a magnetic stirring bar, and then the solution was degassed by three freeze−evacuate−thaw cycles. After the ampule was flame-sealed under vacuum, it was stirred at 60 °C for 24 h. The characteristic pale yellow color remained during the polymerization. The reaction was stopped by rapid cooling with liquid nitrogen. For the determination of the monomer conversion, the ^{1}H NMR spectrum of the polymerization mixture collected just after the polymerization was measured in CD_3OD at room temperature, and the integration of the monomer C=C−*H* resonance at around 5.7 ppm was compared with the sum of N−C−*H* peak intensity of the polymer and the monomer at around 4.2−4.7 ppm. Conversion determined by this method was 91%. After removing the solvents by evaporation, a part of the crude sample was employed for methylation without any purification, which was directly used for the size-exclusion chromatography (SEC) measurements. For the ^{1}H NMR measurement and chain extension experiment, the crude polymer was purified by reprecipitation from a MeOH solution into a large excess of tetrahydrofuran (THF)/hexane (8/2 vol %), and the resulting product was dried under vacuum at room temperature: yield 0.31 g, 80%. The resulting poly(A-Pro-OH) was soluble in basic water (pH = 10), methanol, ethanol, dimethyl sulfoxide (DMSO), DMF, and insoluble in neutral water (pH ≈ 7), acidic water (pH = 1), ethyl acetate, THF, chloroform, diethyl ether, hexane. ^{1}H NMR (CD_3OD): δ 1.2−3.0 (7H, CH and CH$_2$ in the polymer main chain and NCH$_2$C*H$_2$*C*H$_2$*CH in the proline residue), 3.5−3.8 (2H, >NC*H$_2$*−), and 4.4−4.6 ppm (1H, >C*H*COOH).

For SEC measurements, the crude poly(A-Pro-OH)s were modified by methylation of the carboxylic acid groups using trimethylsilyldiazomethane according to a method reported previously with a slight modification.[57,69] In this way, 25 mg of each sample was dissolved in a mixture of THF/methanol (2/1 vol %, to get solubilization at room temperature), overall volume 3.0 mL. The yellow solution of trimethylsilyldiazomethane (0.50 mL, 1.00 mmol, $(CH_3)_3SiCHN_2$/COOH group in poly(A-Pro-OH) = 7/1 molar ratio) was added dropwise at room temperature into the polymer solution. Upon addition, bubbles appeared and the bright yellow solution became instantaneously pale yellow. After the methylation agent was added completely, the solution was stirred for 1 h more at room temperature. After removing the solvents by evaporation, the methylated samples were employed without any purification for the SEC measurements. For the confirmation of the degree of the esterification, the methylated mixture was precipitated from $CHCl_3$ into diethyl ether, then sample was evaluated by ^{1}H NMR spectroscopy in CD_3OD (see Figure S2 in Supporting Information). The degree of esterification was more than 95%, as judged by ^{1}H NMR spectroscopy by comparing the integration of the methyl resonance at around 3.5−4.0 ppm (>NC*H$_2$*−, COOC*H$_3$*) with the intensity of the methine resonance at 4.4−4.6 ppm (>C*H*COO). The poly(A-Pro-OMe) obtained after the methylation was soluble in most organic solvents, such as dichloromethane, acetone, dioxane, DMF, and DMSO, and insoluble in hexane, water in the wide pH ranges (pH = 1, 7, 10). ^{1}H NMR (CD_3OD): δ 1.2−3.0 (7H, C*H* and C*H$_2$* in the polymer main chain, NCH$_2$C*H$_2$*C*H$_2$*CH in the proline residue), 3.5−4.0 (5H, >NC*H$_2$*−, COOC*H$_3$*), and 4.4−4.6 ppm (1H, >C*H*COO). The ^{1}H NMR and FT-IR spectra of poly(A-Pro-OMe) and poly(A-Pro-OH) are shown in Figures S2 and S5 (see Supporting Information).

The theoretical number-average molecular weight upon conversion is defined as follows:

$$M_{\mathrm{n}}(\mathrm{theor}) = \frac{[\mathrm{Monomer}]_0}{[\mathrm{CTA}]_0 + 2f[\mathrm{I}]_0(1 - \mathrm{e}^{-k_{\mathrm{d}}t})} \times M_{\mathrm{Monomer}} \times \mathrm{convn} + M_{\mathrm{CTA}} \quad (1)$$

in which M_{CTA} and M_{Monomer} are the molecular weights of CTA and the monomer, and $[\mathrm{Monomer}]_0$ and $[\mathrm{CTA}]_0$ are the initial concentrations of the monomer and CTA, respectively. The right-hand side of the denominator accounts for the radicals derived from the initiator with an initial concentration $[\mathrm{I}]_0$ at time t and a decomposition rate, k_{d}. The initiator efficiency is represented by f. Note that eq 1 is only effective for the disproportional termination. In an ideal RAFT process, the polymer directly derived from the initiators is considered to be minimal, and thus the second term in the denominator becomes negligible and equation 1 can be simplified to equation 2,[37,38,40]

$$M_{\mathrm{n}}(\mathrm{theor}) = \frac{[\mathrm{Monomer}]_0}{[\mathrm{CTA}]_0} \times M_{\mathrm{Monomer}} \times \mathrm{convn} + M_{\mathrm{CTA}} \quad (2)$$

where M_{CTA} and M_{Monomer} are molecular weights of chain transfer agent and monomer, and $[\mathrm{Monomer}]_0$ and $[\mathrm{CTA}]_0$ are the initial concentrations of monomer and chain transfer agent, respectively.

For the kinetic study, typically a mixed solution of the A-Pro-OH (1.50 g, 8.8 mmol), CTA 2 (41.4 mg, 0.177 mmol), AIBN (5.8 mg, 0.0354 mmol), and dry methanol (6.0 mL) were divided into six glass ampules, and then each solution was degassed by three freeze−evacuate−thaw cycles. After the ampule was sealed by flame under vacuum, it was stirred at 60 °C for the desired time. The reaction was stopped by rapid cooling with liquid nitrogen, and the monomer conversion was determined by the ^{1}H NMR spectrum of the polymerization mixture. The crude poly(A-Pro-OH)s were modified by the methylation, and the resulting poly(A-Pro-OMe)s were used directly for SEC measurements without any purification.

For the polymerization of A-Hyp-OH, typically a mixed solution of A-Hyp-OH (0.167 g, 0.913 mmol), CTA 1 (4.46 mg, 0.0183 mmol), AIBN (1.5 mg, 0.0091 mmol), and dehydrated DMF (0.67 mL) was degassed by three freeze−evacuate−thaw cycles. After the ampule was sealed by flame under vacuum, it was stirred at 60 °C for 24 h. Conversion determined by the integration of the monomer C=C−*H* resonance at around 5.8 ppm compared with the sum of N−C−*H* peak intensity of the polymer and the monomer at around 4.2−4.5 ppm was 87%. The resulting polymer was purified by reprecipitation from a methanol solution into a large excess of acetone. Then the product was freeze-dried from water and finally dried under vacuum at room temperature: yield 0.134 g, 80%. The resulting poly(A-Hyp-OH) was soluble in water, methanol, ethanol, DMSO, and DMF, and insoluble in ethyl acetate, THF, chloroform, dichloromethane, diethyl ether, and hexane. ^{1}H NMR (D_2O): δ 1.2−3.0 (5H, C*H* and C*H$_2$* in the polymer main chain and C*H$_2$* in the proline residue), 3.0−3.8 (2H, >NC*H$_2$*−), and 4.2−4.5 (2H, C*H*OH, NC*H*COOH) ppm.

The methylation of poly(A-Hyp-OH) was also conducted by treating the carboxylic acid groups using trimethylsilyldiazomethane, as mentioned above. The resulting product was finally freeze-dried from water and dried under vacuum at room temperature. The degree of esterification was more than 95%, as judged by ^{1}H NMR spectroscopy (Figure S4 in the Supporting Information) by comparing the integration of the methyl resonance at around 3.5−4.0 ppm (>NCH_2−, $COOCH_3$) with the intensity of the methine resonances at 4.0−4.5 ppm (CHOH, NCHCOO). The existence of hydroxyl group in poly(A-Hyp-OMe) was confirmed by FT-IR measurement (Figure S6 in the Supporting Information). The poly(A-Hyp-OMe) obtained after the methylation was soluble in most organic solvents, such as chloroform, ethanol, methanol, DMF, and water in the wide pH ranges (pH = 1. 7, 10) and insoluble in acetone, ethyl acetate, THF, dichloromethane, diethyl ether, and hexane. ^{1}H NMR (D_2O): δ 1.1−3.0 (5H, CH and CH_2 in the polymer main chain and CH_2 in the proline residue), 3.5−4.0 (5H, >NCH_2−, $COOCH_3$), and 4.0−4.5 (2H, CHOH, NCHCOO) ppm. The ^{1}H NMR and FT-IR spectra of poly(A-Hyp-OMe) and poly(A-Hyp-OH) are shown in Figures S4 and S6 (see Supporting Information).

The copolymerization of A-Hyp-OH and A-Pro-OMe was carried out with AIBN in the presence of CTA 2 in dehydrated methanol using the same procedure mentioned above. After the copolymerization, the product was precipitated in a large excess of diethyl ether/acetone (7/3 vol %) and isolated by filtration. The copolymer composition was determined using ^{1}H NMR spectroscopy by a comparison of peaks associated with the two comonomers. The peak at 3.5−4.0 ppm is attributed the methylene protons (NCH_2) of the both units and the methyl protons of A-Pro-OMe unit, whereas the peaks at 4.2−4.6 ppm correspond to the methine protons of the both units (NCHCOO) and A-Hyp-OH unit (CHOH), as shown in Figure S9 (see Supporting Information). Thus, the comonomer composition can be calculated using the equation 3,

$$\frac{2(x) + 5(1-x)}{2(x) + 1(1-x)} = \frac{\text{integral at 3.5−4.0 ppm}}{\text{integral at 4.2−4.6 ppm}} \qquad (3)$$

where x is the fraction of the A-Hyp-OH and $1 - x$ is the fraction of A-Pro-OMe in the random copolymer.

Instrumentation. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a JEOL JNM-ECX400. Specific rotations ($[\alpha]_D$) were measured on a JASCO DIP-1000 digital polarimeter equipped a sodium lamp as a light source. FT-IR spectra were obtained with a JASCO FT/IR-210 spectrometer.

Number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were estimated by size-exclusion chromatography (SEC) using a Tosoh HPLC HLC-8220 system equipped with refractive index and ultraviolet detectors at 40 °C. The column set was as follows: four consecutive hydrophilic vinyl polymer-based gel columns [TSK-GELs (pore size, exclusion limited molecular weight): α-M (13 μm, >1 × 10^7), α-4000 (10 μm, 4 × 10^5), α-3000 (7 μm, 9 × 10^4), α-2500 (7 μm, 5 × 10^3), 30 cm each] and a guard column [TSK-guard column α, 4.0 cm]. The system was operated at a flow rate of 1.0 mL/min, using DMF containing 10 mM LiBr as an 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 temperature controller system (JASCO EHC-716 and EHC-717). The temperature was increased at a rate of 1.0 °C/min in heating scans between 10 and 60 °C. Elemental analysis was carried out on a Perkin-Elmer 2400ΠCHNS/O analyzer.

Results and Discussion

RAFT Polymerization of A-Pro-OH. We initially investigated the effects of the nature of CTA and CTA/initiator ratio on the homopolymerization of *N*-acryloyl-L-proline (A-Pro-OH). In a previous publication,[57] we demonstrated that methanol was an effective solvent for the controlled radical polymerization of a phenylalanine derivative containing carboxylic acid, *N*-acryloyl-L-phenylalanine, and the polymer with low polydispersity could be obtained, depending on the $[CTA]_0/[AIBN]_0$ ratio and the kind of the solvent. On the basis of the results, we conducted direct polymerization of the unprotected monomer, A-Pro-OH, in methanol at 60 °C using two different CTAs. The results are summarized in Table 1. When the polymerization of A-Pro-OH was conducted using the dithiocarbamate-type CTA (CTA 2) at $[\text{A-Pro-OH}]_0/[\text{CTA 2}]_0/[\text{AIBN}]_0 = 100/2/1$, almost full conversion (>99%, as determined by ^{1}H NMR spectroscopy) was obtained after 24 h. The resulting poly(A-Pro-OH) was soluble in basic water (pH = 10), while being insoluble in neutral water (pH ≈ 7) and acidic water (pH = 1), suggesting that the degree of ionization and water-solubility can be manipulated simply by pH value. For SEC measurement, the resulting poly(A-Pro-OH) was converted into its methyl ester form by treating the carboxylic acid groups using trimethylsilyldiazomethane.[57,69] As verified by ^{1}H NMR analysis of the polymers, the proportion of methyl ester was almost quantitative in all cases. Note that the crude samples were employed for SEC measurements without any purification in both the polymerization and esterification processes. Hence, we can exclude the possibility to lose shorter terminated chains due to slow/ineffective chain transfer. The methylated polymer, poly(*N*-acryloyl-L-proline methyl ester) (poly(A-Pro-OMe)), showed symmetrical unimodal SEC peak (see Figure S7 in Supporting Information) with relatively narrow molecular weight distribution (M_w/M_n = 1.30). The number-average molecular weight of the poly(A-Pro-OMe), measured by a GPC in DMF with 10 mM LiBr, was M_n = 8000, which is comparable to the theoretical value (M_n = 9300) calculated from the monomer/CTA molar ratio and the monomer conversion using eq 2. In contrast, a conventional radical polymerization of A-Pro-OH under the similar conditions in the absence of CTA afforded a high molecular weight homopolymer with high polydispersity (M_n = 93000 and M_w/M_n = 3.34, entry 1). The difference in the molecular weights of the polymers obtained in the presence and absence of CTA under the similar conditions supports the effectiveness of CTA.[70]

Table 1. Effects of Chain Transfer Agent (CTA) and Solvent on Polymerization of *N*-Acryloyl-L-proline (A-Pro-OH) at 60 °C for 24 h[a]

entry	CTA[b]	solvent	convn[c] (%)	$M_{n,\text{theory}}$[d]	$M_{n,\text{SEC}}$[e]	$M_w/M_{n,\text{SEC}}$[e]
1		MeOH	97	-	93000	3.34
2	CTA 1	MeOH	72	6800	7600	1.26
3	CTA 1	EtOH	47	4200	5500	1.29
4	CTA 1	DMF	89	7800	7500	1.25
5	CTA 2	MeOH	>99	9300	8000	1.30
6	CTA 2	EtOH	>99	9300	6900	1.29
7	CTA 2	DMF	>99	9300	6400	1.25

[a] $[\text{AIBN}]_0/[\text{CTA}]_0/[\text{A-Pro-OH}]_0 = 1/2/100$, monomer concentration = 0.25 g/mL, where AIBN = 2,2′-azobis(isobutyronitrile), A-Pro-OH = *N*-acryloyl-L-proline. [b] CTA 1 = benzyl dithiobenzoate, CTA 2 = benzyl 1-pyrrolecarbodithioate (see Scheme 2). [c] Calculated by ^{1}H NMR in CD_3OD. [d] The theoretical molecular weight ($M_{n,\text{theory}}$) = (MW of A-Pro-OMe) × $[\text{A-Pro-OH}]_0/[\text{CTA}]_0$ × convn + (MW of CTA), A-Pro-OMe = *N*-acryloyl-L-proline methyl ester. [e] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr).

Solvent effects on radical polymerization originate from various factors, such as polarity, interaction between the polymer (or monomer) and the solvent, solvation of a transition state (or intermediate) that may have polar characteristics, and complexation between the propagating radical (or monomer) and the solvent.[71] The solvent may also affect the diffusion-controlled termination, and thus the termination rate coefficient may be strongly altered. The polymerizations of A-Pro-OH with AIBN in the presence of CTA 2 in ethanol and DMF at 60 °C produced the polymers quantitatively after 24 h. Unimodal SEC traces with no evidence of high molecular weight species were obtained, and the molecular weight distributions were relatively narrow (M_w/M_n = 1.25 and 1.29). In the case of the polymerization in DMF, which is a strong proton-accepting solvent and is known to act as a complex-breaking (hydrogen bond-breaking) solvent, the number-average molecular weight of the methylated polymer ($M_n = 6400$) is slightly lower than the theoretical value ($M_n = 9300$). Here, we cannot exclude the possibility of contributing to the chains derived from the initiators at $[\mathrm{CTA}]_0/[\mathrm{AIBN}]_0 = 2/1$. If the AIBN-derived chains are taken into account in this system, a rough estimation suggests that $M_{n,\mathrm{theory}}$ corresponds to 6200 (calculated form eq 1 with $k_d = 2.5 \times 10^{-5}\ \mathrm{s}^{-1}$,[72] $f = 0.6$, at 60 °C, complete conversion for 24 h reaction), instead of $M_{n,\mathrm{theory}} = 9300$ (calculated from eq 2 without consideration of AIBN-derived chains). Since the molecular weight distribution remains low despite the high conversion, however, the contribution of the AIBN-derived chains should be minimal.

The polymerization of A-Pro-OH in methanol with CTA 1 under the same conditions produced the polymer with 72% conversion. Although the polydispersity remains low ($M_w/M_n = 1.26$, entry 2 in Table 1), the resulting polymer shows SEC peak with a broad shoulder at high molecular weight region, (see Figure S7 in the Supporting Information). This is most probably attributed to species arising from bimolecular termination reactions of the growing polymer chains. The polymerizations in other polar solvents, ethanol and DMF, showed the same tendencies, in which the polymerizations were relatively slow and the resulting polymers had bimodal distributions. These results suggest that the polymerization of A-Pro-OH with CTA 1 is much slower than that with CTA 2 at 60 °C, regardless of the solvents used for the polymerizations. Similar phenomenon was observed in our previous works concerning RAFT polymerizations of *N*-acryloyl-L-phenylalanine methyl ester[56] and *N*-acryloyl-L-phenylalanine.[57] Since the leaving group (benzyl radical) of CTA 1 is the same to that of CTA 2, the slow polymerization with CTA 1 should be related to the slower fragmentation rate from the phenyl substituted intermediate radical than that from pyrrole one and/or fast addition rate of expelled radical to the thiocarbonyl group. The transfer constants of a series of benzyl thiocarbonylthio compounds of general structures, $\mathrm{Z{-}C({=}S)S{-}CH_2Ph}$, in styrene polymerization have been reported, and it was demonstrated that the transfer constant with CTA 1 (Z = Ph, $C_{tr} = 26$) is apparently larger than that with CTA 2 (Z = pyrrole, $C_{tr} = 9$).[70] The high transfer constant for CTA 1 reflects the stability of the intermediate radicals, because the value directly leads to increased addition rates.

The CTA/initiator ratio acts frequently as a critical role in determining the overall success of a RAFT polymerization with respect to control over the molecular weight and molecular weight distribution with a sufficiently fast polymerization rate.[38,70] In general, higher CTA/initiator ratios (lower concentration of the initiator) may afford better overall control of the polymerization, due to a decrease in the number of radicals available for unfavorable side reactions, but often provide a longer polymerization time. Whereas, lower CTA/initiator ratios often result in faster polymerization rate and may be less controlled, suggesting a potential drawback for achieving controlled polymerization system. In order to evaluate the effect, we conducted the polymerization of A-Pro-OH at 60 °C for 24 h at different $[\mathrm{CTA}]_0$:$[\mathrm{AIBN}]_0$ ratios between 2 and 10, keeping the monomer-to-chain transfer agent ratio at a constant value of $[\text{A-Pro-OH}]_0/[\mathrm{CTA}]_0 = 50/1$. Under these conditions, the concentrations of A-Pro-OH and CTA are constant, and the initiator concentration is substantially different. The results are summarized in Table 2. The polymerizations with CTA 2 in methanol produced the polymers having relatively narrow molecular weight distributions ($M_w/M_n = 1.23{-}1.32$), irrespective of the concentration ratio of CTA to initiator. In the cases, only slight influence of the CTA/initiator ratio was observed on the monomer conversion, molecular weights, and molecular weight distribution. When the polymerization was carried out with CTA 2 in DMF at 60 °C, the monomer conversion decreased gradually from 99% to 85% as [CTA]/[AIBN] ratio increased from 2 to 10. The molecular weight distributions of the polymers obtained at $[\mathrm{CTA}]_0/[\mathrm{AIBN}]_0 = 5$ and 10 are slightly broader ($M_w/M_n = 1.37$ and 1.34), compared with that at $[\mathrm{CTA}]_0/[\mathrm{AIBN}]_0 = 2/1$ ($M_w/M_n = 1.25$). Generally, decreasing initiator concentration leads to an improvement of the control of the polymerization, since termination reactions will be disfavored. The narrow molecular weight distribution is apparently due to rapid establishment of the "pre-equilibrium", which involves the consumption of CTA and reversible fragmentation of intermediate to produce reinitiating $\mathrm{R}^\bullet$ fragment, efficient reinitiation from the $\mathrm{R}^\bullet$ fragment, and attainment of the so-called "main-equilibrium" in which the population of dormant chains and/or intermediate radicals is much higher than the total number of propagating chains.[36] In the cases of the polymerizations with CTA 1 in methanol, a drastic decrease in the monomer conversion was observed at higher $[\mathrm{CTA}]_0/[\mathrm{AIBN}]_0$ ratio (convn < 5% at $[\mathrm{CTA}]_0/[\mathrm{AIBN}]_0 = 5$).

Table 2. Effect of Chain Transfer Agent/Initiator Molar Ratio on Polymerization of *N*-Acryloyl-L-proline (A-Pro-OH) Using AIBN at 60 °C for 24 h in Different Solvents[a]

entry	CTA[b]	solvent	$[\mathrm{CTA}]_0/[\mathrm{AIBN}]_0$	convn[c] (%)	$M_{n,\mathrm{theory}}$[d]	$M_{n,\mathrm{SEC}}$[e]	$M_w/M_{n,\mathrm{SEC}}$[e]
1	CTA 1	MeOH	2	72	6800	7600	1.26
2	CTA 1		3	76	7200	8300	1.20
3	CTA 1		5	<5			
4	CTA 2	MeOH	2	>99	9300	8000	1.30
5	CTA 2		5	96	9000	8300	1.25
6	CTA 2		10	92	8700	8600	1.23
7	CTA 2	DMF	2	99	9300	6400	1.25
8	CTA 2		5	99	9300	5800	1.37
9	CTA 2		10	85	8000	5600	1.34

[a] $[\text{A-Pro-OH}]_0/[\mathrm{CTA}]_0 = 50$, monomer concentration = 0.25 g/mL, where AIBN = 2,2′-azobis(isobutyronitrile), A-Pro-OH = *N*-acryloyl-L-proline. [b] CTA 1 = benzyl dithiobenzoate, CTA 2 = benzyl 1-pyrrolecarbodithioate (see Scheme 2). [c] Calculated by ^{1}H NMR in $\mathrm{CD_3OD}$. [d] The theoretical molecular weight ($M_{n,\mathrm{theory}}$) = (MW of A-Pro-OMe) × $[\text{A-Pro-OH}]_0/[\mathrm{CTA}]_0$ × conv. + (MW of CTA), A-Pro-OMe = *N*-acryloyl-L-proline methyl ester. [e] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr).

Aiming to control the molecular weights, we further examined the polymerization of A-Pro-OH in methanol at 60 °C for 24 h at different $[\mathrm{M}]_0$:$[\mathrm{CTA}]_0$ ratios between 50 and 200, keeping the chain transfer agent-to-initiator ratio at a constant value of $[\mathrm{CTA\ 2}]_0/[\mathrm{AIBN}]_0 = 5/1$. Under the conditions, the conversions determined by ^{1}H NMR were 90−96% in all cases (see Supporting Information). Figure 1a shows the relation of the molecular weight and polydispersity of the methylated samples, poly(A-Pro-OMe)s, with $[\mathrm{M}]_0$:$[\mathrm{CTA}]_0$ ratio for the polymerization. The number-average molecular weight increases with the ratio without significant change of the polydispersity ($M_w/M_n = 1.24{-}1.32$), indicating a feasibility to control the molecular weights by the ratio. In all cases the SEC traces are unimodal with no evidence of high molecular weight species, as can be seen in Figure 1b. Note that a linear increase of the molecular weight with the $[\mathrm{M}]_0$:$[\mathrm{CTA}]_0$ ratio could be obtained only when the monomer conversions are substantially the same in all cases. Nevertheless, these results suggest that the amino acid−based polyelectrolytes, poly(A-Pro-OH)s, having predetermined molecular weights and low polydispersities can be easily obtained by direct RAFT polymerization of the carboxylic acid-containing monomer, A-Pro-OH, without protecting chemistry.

NMR technique was used to determine the chain-end structure and absolute molecular weights of poly(A-Pro-OH)s. The ^{1}H NMR spectrum of the poly(A-Pro-OH) obtained at $[\text{A-Pro-OH}]_0/$

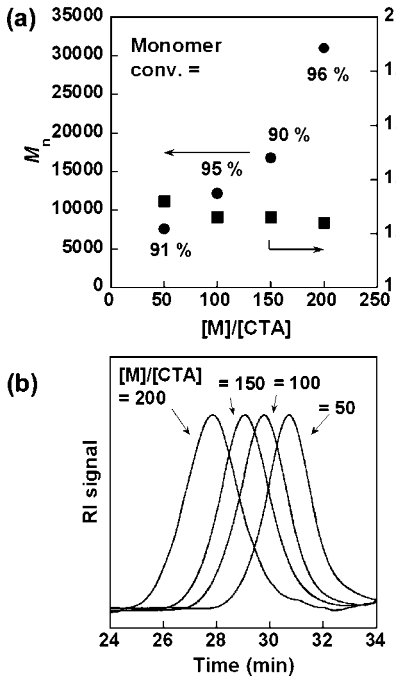


Figure 1. (a) Dependence of number-average molecular weight and molecular weight distribution of the methylated poly(A-Pro-OH)s on the initial monomer-to-chain transfer agent ratio, $[M]_0$:$[CTA]_0$. Monomer conversion = 90–96%, as shown inside the figure. The polymerization of *N*-acryloyl-L-proline (A-Pro-OH) was conducted at different $[M]_0$:$[CTA]_0$ ratios with 2,2′-azobis(isobutyronitrile) (AIBN) in the presence of benzyl 1-pyrrolecarbodithioate (CTA 2, see Scheme 2) in methanol at 60 °C. Monomer concentration = 0.25 g/mL. $[CTA\ 2]_0/[AIBN]_0 = 5/1$. See Table S1 (Supporting Information) for detailed polymerization results and conditions. (b) SEC traces of the methylated poly(A-Pro-OH)s.

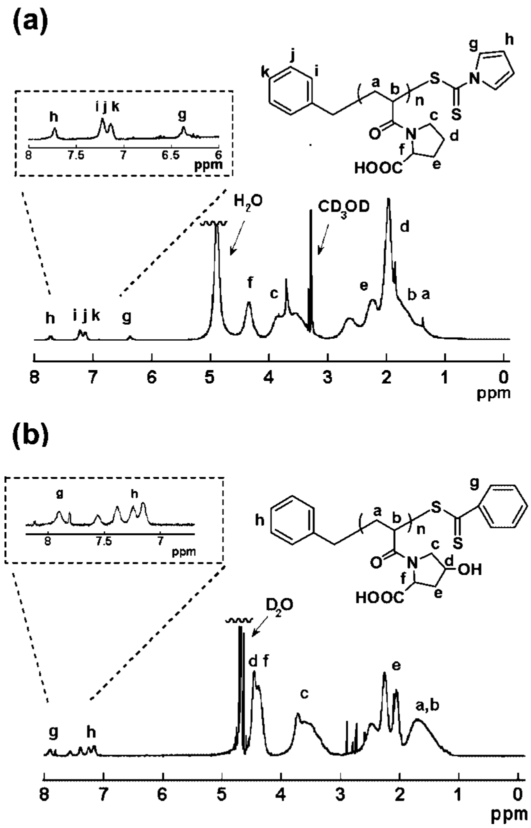


Figure 2. ^{1}H NMR spectra of (a) the dithiocarbamate-terminated poly(A-Pro-OH) (CD_3OD, $M_{n,GPC} = 4600$, $M_w/M_n = 1.24$) and (b) the dithiobenzoate-terminated poly(A-Hyp-OH) (D_2O, $M_{n,GPC} = 5700$, $M_w/M_n = 1.33$).

$[CTA\ 2]_0/[AIBN]_0 = 125/5/1$ is presented in Figure 2a. The characteristic peaks at 1.0–2.8 (CH_2CH in the backbone, $NCH_2CH_2CH_2CH$), 3.2–4.0 (NCH_2), and 4.2–4.6 ($NCHCOO$) ppm are clearly seen, which are attributed to poly(A-Pro-OH) main chain. In addition to these peaks, aromatic peaks are clearly visible at 6.0–8.0 ppm, which correspond to the benzyl and pyrrole fragments at chain ends of the polymer. This is an indication that the polymer chain end is capped with the fragments of CTA as expected according to the general mechanism of the RAFT process. The molecular weight of the polymer chain can be calculated by comparing the integrals of peaks for the chain-end protons to those of the main-chain protons. The molecular weight of poly(A-Pro-OH) calculated by comparison of the area of the peak at 4.2–4.6 ppm corresponding to the methine proton adjacent to an carboxyl group (peak “f” in Figure 2a) in A-Pro-OH repeating units to the peak at 7.8–7.9 ppm (peak “h”) corresponding to two protons of the end group (Z-group) is $M_n = 6800$ in the carboxylic acid form ($M_{n,\ NMR} = 6300$ in the methylated form). The molecular weight determined by NMR is comparable to the methylated sample ($M_{n,GPC} = 4600$) obtained from GPC using polystyrene standards and the theoretical value ($M_n = 4600$) calculated using the equation 2. The molecular weight can be also calculated by using another end group (R-group), peaks “i, j, and k” at 7.0–7.3 ppm, corresponding to the benzyl fragment ($M_{n,\ NMR} = 5300$ in the methylated form). This spectroscopic method was also valid for the poly(A-Pro-OH) having relatively higher molecular weights ($M_{n,NMR} = 8900$ (methylated form), $M_{n,GPC} = 8300$, and $M_{n,theory} = 8600$,) obtained at $[A\text{-}Pro\text{-}OH]_0/[CTA\ 2]_0/[AIBN]_0 = 250/5/1$. However, it was hard to evaluate the higher molecular weights ($M_n > 10000$) by using this spectroscopic method, because of low signal-to-noise ratio of the peak of the chain end groups.

The controlled/living character of this process was investigated by performing kinetic investigations at 60 °C. Figure 3a shows the variation in $\ln([M]_0/[M]_t)$ versus polymerization time for the polymerizations of A-Pro-OH in methanol with AIBN in the presence of CTA 2. When the reaction was conducted at $[A\text{-}Pro\text{-}OH]_0/[CTA\ 2]_0/[AIBN]_0 = 1000/5/1$, which corresponds to [M] = 1.48 mol/L, [CTA] = 0.0073 mol/L, and [AIBN] = 0.00148 mol/L, an almost full conversion (96%) was reached after 24 h. A linear first-order kinetic plot is seen until the last stage of the polymerization (Figure 3a). As shown in Figure 3b, a linear increase in the number-average molecular weight with conversion reveals a constant number of propagating chains throughout the polymerization. The SEC traces (refractive index) of the methylated samples, poly(A-Pro-OMe)s, obtained at different reaction times are shown in Figure 3c. A progressive increase in the molar mass with conversion with narrow unimodal SEC peaks ($M_w/M_n = 1.24$–1.34) is clearly seen, as normally evidenced for a controlled/living polymerization. Note that SEC measurement was conducted after the esterification without any purification process.

The polymerization behavior was also compared at different targeted polymerization degrees, $[A\text{-}Pro\text{-}OH]/[CTA\ 2] = 50$ and 200, keeping the chain transfer agent-to-initiator ratio at a constant value of $[CTA\ 2]_0/[AIBN]_0 = 5$ at 60 °C. In the case of the polymerization in methanol at $[A\text{-}Pro\text{-}OH]_0/[CTA\ 2]_0 = 50$, the conversion reaches a plateau value after 10 h. Nevertheless, the number-average molecular weight increases with the conversion, maintaining low polydispersity ($M_w/M_n = 1.26$–1.35), as shown in Figure 3b. The molecular weights of poly(A-Pro-OMe)s obtained at $[M]_0/[CTA]_0 = 200$ are reasonably higher than those at $[M]_0/[CTA]_0 = 50$. The linear relationships

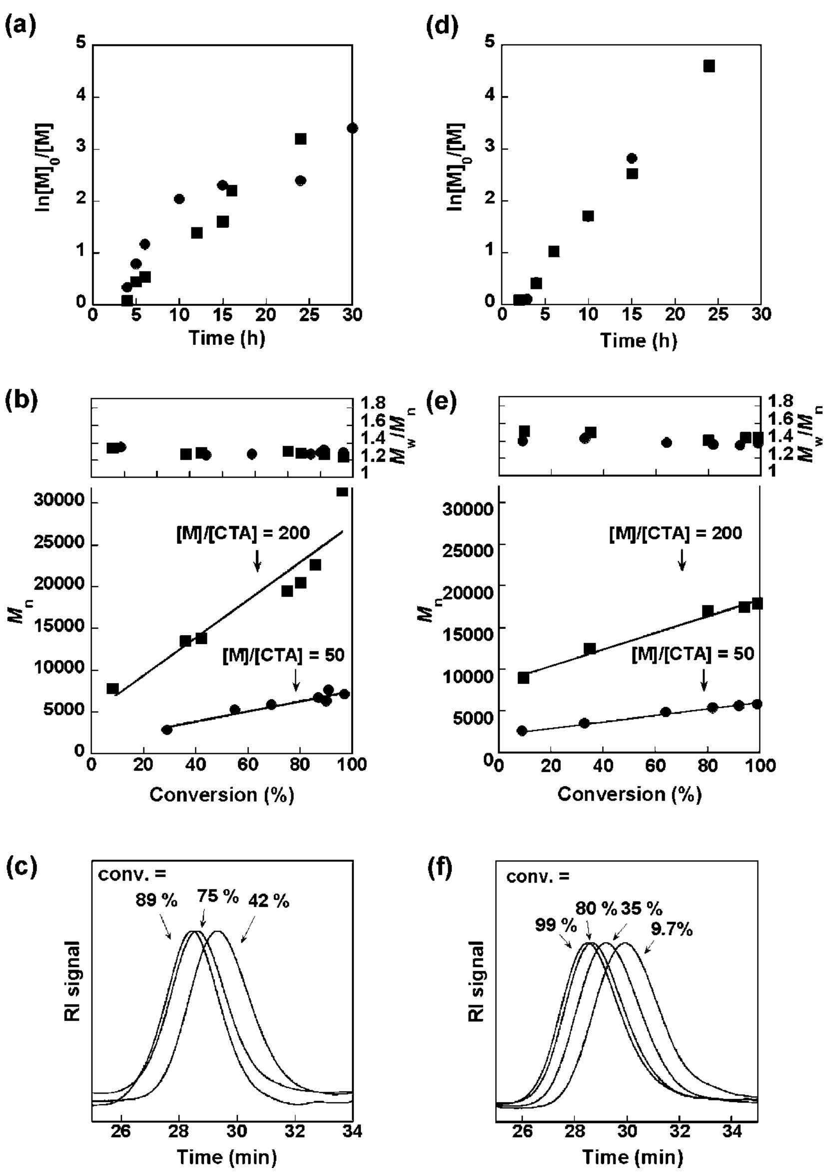


Figure 3. (a, d) First-order kinetic plots for the polymerization of *N*-acryloyl-L-proline (A-Pro-OH) with 2,2′-azobis(isobutyronitrile) (AIBN) in the presence of benzyl 1-pyrrolecarbodithioate (CTA 2, see Scheme 2) at 60 °C. Monomer concentration = 0.25 g/mL. [A-Pro-OH]$_0$/[CTA 2]$_0$ = 50 (circles) and 200 (squares), which are initial monomer-to-chain transfer agent ratios and correspond to the targeted degrees of polymerizations. [CTA 2]$_0$/[AIBN]$_0$ = 5. Solvent = (a−c) methanol and (d−f) DMF. (b, e) Number-average molecular weight and polydispersity as a function of conversion. (c, f) Evolution of SEC traces of the methylated samples with conversion ([A-Pro-OH]$_0$/[CTA 2]$_0$ = 200).

between the molecular weight and conversion with low polydispersities observed in both [M]$_0$/[CTA]$_0$ ratios suggest that the controlled character of the polymerization was achieved, regardless of the targeted polymerization degrees.

The polymerization kinetics of A-Pro-OH was also investigated in DMF under the same conditions ([A-Pro-OH]/[CTA 2] = 50 and 200, [CTA 2]/[AIBN] = 5). The linear pseudofirst order kinetic plots are seen, regardless of the monomer-to-chain transfer agent ratio (Figure 3d). As can be seen in Figure 3, parts a and d, the polymerization rates in DMF are slightly faster than those in methanol. In all cases, induction periods of less than 5 h are visible in the pseudofirst-order kinetic plots. Similar induction period was also observed in RAFT polymerizations of *N*-acryloyl-L-phenylalanine methyl ester,[56] *N*-acryloyl-L-phenylalanine,[57] and A-Pro-OMe.[59] Although a number of possible explanations have been suggested, there is still an ongoing debate on the mechanism that causes the inhibition.[41] The "pre-equilibrium", which involves consumption of CTA and reversible fragmentation of intermediate to produce reinitiating R$^\bullet$ fragment, is correlated closely with the induction period. Possible explanations include slow conversion of the initial CTA, slow fragmentation of the initiating leaving group radical, slow reinitiation by the expelled radical, increased stability of the intermediate radical (with and without intermediate radical termination), tendency of the expelled radical to add to the CTA rather than to monomer, and impurities in the CTA.[73–77] Figure 3e shows the evolutions of M_n and M_w/M_n with conversion for A-Pro-OH during the polymerizations in DMF. A linear increase in the number-average molecular weights, M_n, with conversion is seen, and the polydispersity indices (M_w/M_n) for all samples range between 1.24 and 1.40, regardless of the monomer-to-chain transfer agent ratio. These results suggest that the polymerization proceeds in a controlled fashion. In both solvents (methanol and DMF), positive intercepts of the M_n vs conversion plot are observed clearly at [M]$_0$/[CTA]$_0$ = 200. Similar behavior was also found in other RAFT polymerization systems,[78] and such positive intercept was reported to occur when the rate of polymerization is greater than the rate of addition to CTA.[73]

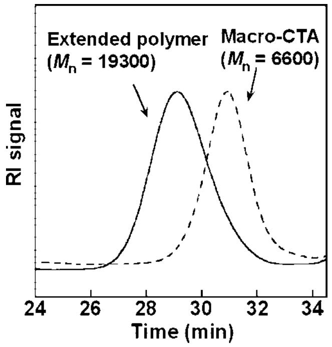


Figure 4. SEC traces of the methylated samples of the parent poly(A-Pro-OH) macro-CTA (dotted trace, M_n = 6600, M_w/M_n = 1.23, conversion = 80%) obtained at [A-Pro-OH]$_0$/[CTA 2]$_0$/[AIBN]$_0$ = 250/5/1 and the chain extended polymer (solid trace, M_n = 19300, M_w/M_n = 1.43, yield = 34%) obtained after the polymerization at [A-Pro-OH]$_0$/[macro-CTA]$_0$/[AIBN]$_0$ = 1000/5/1 for 24 h.

An important criterion of the controlled/living character of the polymerization is the successful extension of a chain from a preformed polymer chain as a macro-CTA. To investigate this point, the dithiocarbamate-terminated poly(A-Pro-OH) (M_n = 6600, M_w/M_n = 1.23, conversion = 80%), which was independently prepared by the polymerization at [A-Pro-OH]$_0$/[CTA 2]$_0$/[AIBN]$_0$ = 250/5/1 in methanol at 60 °C for 24 h, was employed as a macro-CTA for the chain extension experiment. After the chain extension, the resulting product was analyzed by SEC, and compared to the original macro-CTA. When the chain extension was performed at 60 °C at the ratio [A-Pro-OH]$_0$/[macro-CTA]$_0$/[AIBN]$_0$ = 1000/5/1, the polymer yield recovered by precipitation in THF/hexane (8/2 volume ratio) was 34% after 24 h. Figure 4 shows clear shift in the SEC trace toward higher molecular weight region after the chain extension, and a symmetrical SEC peak is seen (M_w/M_n = 1.43). These results suggest that most of the chain ends of the poly(A-Pro-OH) are functionalized with dithiocarbamate end group, which can be used as a macro-CTA for further chain extension reactions.

RAFT Polymerization of A-Hyp-OH. For the controlled synthesis of amino acid−based polymer having higher water solubility and hydrophilicity, we conducted RAFT polymerization of an acrylamide containing hydroxyproline moiety, *N*-acryloyl-4-*trans*-hydroxy-L-proline (A-Hyp-OH), which has a carboxylic acid and a hydroxyl group in the monomer unit. Initially, we investigated the effects of the nature of CTA and solvent on the polymerization of A-Hyp-OH (monomer concentration = 0.25 g/mL) with AIBN at 60 °C in the presence and absence of CTA. The results are summarized in Table 3. When free radical polymerization of A-Hyp-OH was conducted with AIBN, a homogeneous system could be achieved in DMF, whereas the polymer precipitation occurred in methanol. In the case of the polymerization with CTA 2 at [M]$_0$/[CTA]$_0$/[AIBN]$_0$ = 200/2/1, almost full conversion was obtained after 24 h in DMF. While, the polydispersity index of the product prepared by the direct polymerization, followed by methylation, was relatively broad (M_w/M_n = 1.43). The products having lower polydispersity (M_w/M_n = 1.33) were obtained by the polymerization of A-Hyp-OH with CTA1 in DMF and ethanol, and the number-average molecular weights of the methylated polymers were comparable to the theoretical values. The resulting poly(A-Hyp-OH) showed good solubility in water, independent of the pH value.

The polymerization of A-Hyp-OH was investigated in DMF and ethanol at 60 °C at different [CTA 1]$_0$:[AIBN]$_0$ ratios between 2 and 10, keeping the monomer-to-chain transfer agent at a constant value of [M]$_0$/[CTA]$_0$ = 50/1. As shown in Table 4, the monomer conversion decreased from 87% to 42% when [CTA]/[AIBN] ratio increased from 2 to 5 for the polymerization in DMF for 24 h. In the cases of the polymerizations at [CTA]/[AIBN] = 5 and 10, longer polymerization time led to increase in the conversion, achieving 96% and 74% conversions after 48 h, respectively. Interestingly, no significant influence on the molecular weight distribution was observed, and the resulting polymers showed symmetrical unimodal SEC peaks without shoulders and tailing (M_w/M_n = 1.33−1.38), regardless of the [CTA]/[AIBN] ratio. In all cases, the number-average molecular weights of the poly(A-Hyp-OMe)s, measured by GPC in DMF with 10 mM LiBr, are comparable to the theoretical values. Similar tendencies were observed in direct RAFT polymerization of A-Hyp-OH in ethanol. Relatively narrow polydispersities products (M_w/M_n = 1.31−1.35) with reasonable monomer conversions were obtained, regardless of the chain transfer agent-to-initiator ratios ([CTA]/[AIBN] = 2 for 24 h and [CTA]/[AIBN] = 5−10 for 48 h).

Table 3. Effects of Chain Transfer Agent (CTA) and Solvent on Polymerization of *N*-Acryloyl-4-*trans*-hydroxy-L-proline (A-Hyp-OH) at 60 °C for 24 h[a]

entry	CTA[b]	solvent	convn[c] (%)	$M_{n,theory}$[d]	$M_{n,SEC}$[e]	$M_w/M_{n,SEC}$[e]
1[f]		MeOH	99		89600	3.85
2		DMF	>99		23300	2.93
3	CTA 1	DMF	87	9500	9400	1.33
4	CTA 1	EtOH	73	8000	9000	1.33
5	CTA 2	DMF	>99	10800	10200	1.43

[a] [AIBN]$_0$/[CTA]$_0$/[A-Hyp-OH]$_0$ = 1/2/100, monomer concentration = 0.25 g/mL, where AIBN = 2,2′-azobis(isobutyronitrile), A-Hyp-OH = *N*-acryloyl-4-trans-hydroxy-L-proline. [b] CTA 1 = benzyl dithiobenzoate, CTA 2 = benzyl 1-pyrrolecarbodithioate (see Scheme 2). [c] Calculated by ^{1}H NMR in D_2O. [d] The theoretical molecular weight ($M_{n,theory}$) = (MW of A-Hyp-OMe) × [A-Hyp-OH]$_0$/[CTA]$_0$ × convn + (MW of CTA), A-Hyp-OMe = *N*-acryloyl-4-trans-hydroxy-L-proline methyl ester. [e] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr). [f] Polymer precipitation during the polymerization.

Table 4. Effect of Chain Transfer Agent/Initiator Molar Ratio on Polymerization of *N*-Acryloyl-4-trans-hydroxy-L-proline (A-Hyp-OH) with AIBN in the Presence of Benzyl dithiobenzoate (CTA 1) at 60 °C in Different Solvents [a]

entry	solvent	time (h)	[CTA]/[I]	convn[b] (%)	$M_{n,theory}$[c]	$M_{n,SEC}$[d]	$M_w/M_{n,SEC}$[d]
1	EtOH	24	2	73	8000	9000	1.33
2	EtOH	24	5	38	4300	5800	1.35
3	EtOH	48	5	97	10600	11000	1.35
4	EtOH	48	10	91	9900	9900	1.31
5	DMF	24	2	87	9500	9400	1.33
6	DMF	24	5	42	4700	6500	1.38
7	DMF	48	5	96	10500	11100	1.36
8	DMF	48	10	74	8100	9200	1.36

[a] [A-Hyp-OH]$_0$/[CTA]$_0$ = 50, monomer concentration = 0.25 g/mL, where AIBN = 2,2′-azobis(isobutyronitrile), A-Hyp-OH = *N*-acryloyl-4-trans-hydroxy-L-proline. [b] Calculated by ^{1}H NMR in D_2O. [c] The theoretical molecular weight ($M_{n,theory}$) = (MW of A-Hyp-OMe) × [A-Hyp-OH]$_0$/[CTA]$_0$ × conv. + (MW of CTA), A-Hyp-OMe = *N*-acryloyl-4-trans-hydroxy-L-proline methyl ester. [d] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr).

With a view to preparing poly(A-Hyp-OH)s over a wide range of molar mass, the polymerization of A-Hyp-OH was conducted in DMF at different [A-Hyp-OH]$_0$/[CTA 1]$_0$ ratios between 50 and 200, while the AIBN/CTA molar ratio was held constant at 1/2. Under the conditions, the conversions determined by ^{1}H NMR were 87−97% in all cases. As shown in Figure 5a, the number-average molecular weight of the poly(A-Hyp-OMe)s increases with the [M]/[CTA] ratio, indicating the feasibility of controlling the molecular weights. In all cases, the SEC traces are unimodal with no evidence of high molecular weight species (Figure 5b). The molecular weight distributions remained relatively low (M_w/M_n = 1.33) until the [M]/[CTA] ratio of 100,

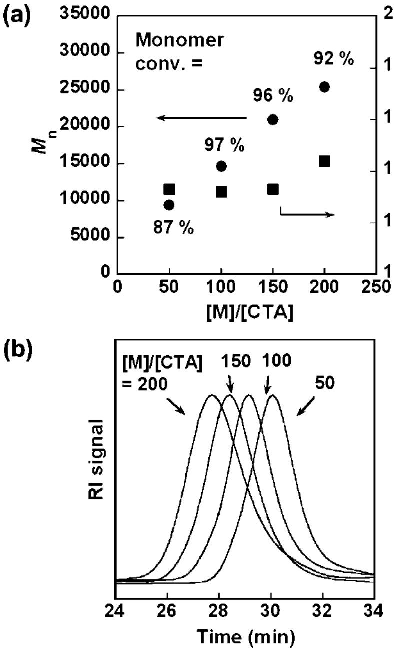


Figure 5. (a) Dependence of number-average molecular weight and molecular weight distribution of the methylated poly(A-Hyp-OH)s on the initial monomer-to-chain transfer agent ratio, $[M]_0$:$[CTA]_0$. Monomer conversion = 87−97%, as shown inside the figure. The polymerization of *N*-acryloyl-4-*trans*-hydroxy-L-proline (A-Hyp-OH) was conducted at different $[M]_0$:$[CTA]_0$ ratios with 2,2′-azobis(isobutyronitrile) (AIBN) in the presence of benzyl dithiobenzoate (CTA 1, see Scheme 2) in DMF at 60 °C. Monomer concentration = 0.25 g/mL. $[CTA\ 1]_0/[AIBN]_0 = 2/1$. See Table S2 (Supporting Information) for detailed polymerization results and conditions. (b) SEC traces of the methylated poly(A-Hyp-OH)s.

while relatively broader polydispersities products (M_w/M_n = 1.41−1.44) with reasonable monomer conversions were obtained, when the polymerization were conducted at higher monomer-to-chain transfer agent ratios ([M]/[CTA] = 150 and 200 (see Table S2 in Supporting Information). This may be due to relatively high molecular weights of the resulting products with increased viscosities at the end of the polymerization, even if the viscosity is low enough to ensure an efficient stirring up under the conditions. Nevertheless, these results suggest the formation of the water-soluble poly(A-Hyp-OH)s, with relatively low polydispersities and number-average molecular weights between 8200 and 22,100 in the carboxylic acid forms (9400 and 25400 in the methylated forms), which can be predetermined by the ratio of concentrations of the reacted monomer to introduced CTA.

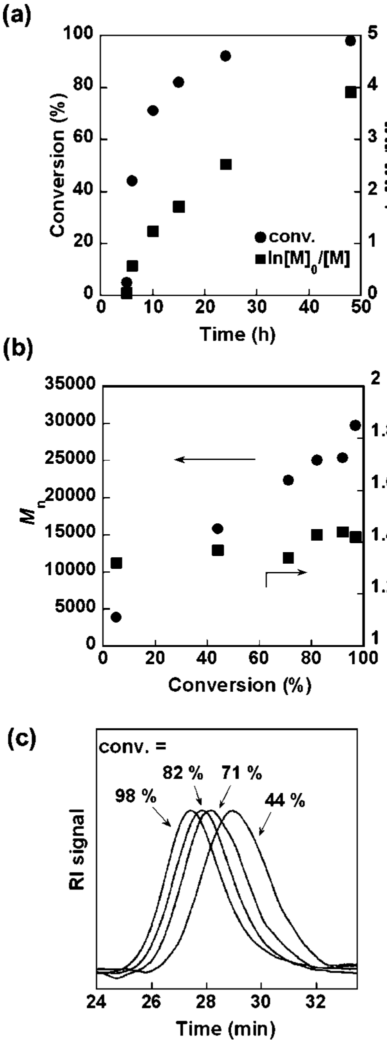


Figure 6. (a) Time-conversion (circles) and first-order kinetic (squares) plots for the polymerization of *N*-acryloyl-4-*trans*-hydroxy-L-proline (A-Hyp-OH) with 2,2′-azobis(isobutyronitrile) (AIBN) in the presence of benzyl dithiobenzoate (CTA 1, see Scheme 2) in DMF at 60 °C. Monomer concentration = 0.25 g/mL. $[A\text{-}Hyp\text{-}OH]_0/[CTA\ 1]_0 = 200$. $[CTA\ 1]_0/[AIBN]_0 = 2$. (b) Number-average molecular weight (circles) and polydispersity (squares) as a function of conversion. (c) Evolution of SEC traces of the methylated sample with conversion.

The polymerization of A-Hyp-OH in the presence of CTA 1 was also investigated in DMF at 60 °C at $[A\text{-}Hyp\text{-}OH]_0/[CTA\ 1]_0/[AIBN]_0 = 400/2/1$. The time-conversion and the pseudo-first-order kinetic plots are shown in Figure 6a. More than 80% conversion is reached within 15 h and the curved semilogarithmic plot is seen. Similar conversion "plateau" was also reported on RAFT polymerization of bulky monosubstituted acrylamides.[57,79] The first-order kinetic plot is considered to be linear only if the kinetics is first-order with respect to the monomer and the concentration of active species remains constant. Since there is no reason to suspect a higher order dependence of the polymerization rate on monomer conversion, the concentration of radicals are not constant via irreversible termination reactions under the conditions. An induction period is also seen in the pseudofirst-order kinetic plot. The induction period roughly estimated simply by extrapolating the linear part of the curve to the time axis is about 5 h. Nevertheless, the linear relationship between the M_n and the conversion with maintaining low polydispersity was observed at 60 °C in DMF at $[CTA\ 1]_0$:$[AIBN]_0$ = 2, as shown in Figure 6b, suggesting negligible irreversible transfer reactions. Symmetrical unimodal SEC peaks were observed for the polymers obtained even at higher conversion (>80%, Figure 6c), and the polydispersity indices (M_w/M_n) for all samples ranged between 1.34 and 1.44. These results suggest that the polymerization of A-Hyp-OH mediated by CTA 1 proceeds in controlled manner in DMF at 60 °C under the conditions used in this study, even if the monomer has a carboxylic acid and a hydroxyl group without protecting chemistry.

Chain-end structure of the resulting poly(A-Hyp-OH) was investigated by ^1H NMR analysis and chain extension experiment. Figure 2b shows the ^1H NMR spectrum (D_2O) of the poly(A-Hyp-OH) obtained at $[A\text{-}Hyp\text{-}OH]_0/[CTA\ 1]_0/[AIBN]_0 = 50/2/1$. In addition to the characteristic peaks attributed to poly(A-Hyp-OH) main chain, the aromatic peaks are clearly visible at 6.0−8.0 ppm, which correspond to the benzyl and phenyl fragments at chain ends of the polymer. The molecular weight of poly(A-Hyp-OH) calculated by comparison of the area of the peak at 3.0−4.0 ppm corresponding to the methylene protons adjacent to a nitrogen atom (peak "c" in Figure 2b) in

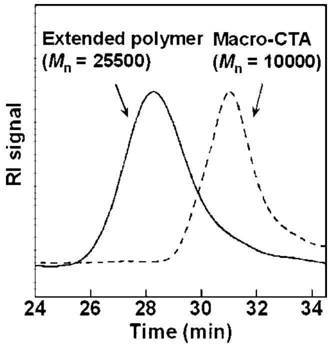


Figure 7. SEC traces of the methylated samples of the parent poly(A-Hyp-OH) macro-CTA (dotted trace, M_n = 10000, M_w/M_n = 1.30, conversion = 89%) obtained at $[\text{A-Hyp-OH}]_0/[\text{CTA 1}]_0/[\text{AIBN}]_0$ = 100/2/1 and the chain extended polymer (solid trace, M_n = 25500, M_w/M_n = 1.51, yield = 64%) obtained after the polymerization at $[\text{A-Hyp-OH}]_0/[\text{macro-CTA}]_0/[\text{AIBN}]_0$ = 300/2/1 for 48 h.

A-Hyp-OH repeating units to the peak at 7.8−8.2 ppm (peak "g") corresponding to two protons of the end group (Z-fragment) is M_n = 5600 in the carboxylic acid form (M_n = 6000 in the methylated form). The value determined by ^{1}H NMR spectroscopy is comparable to that of the methylated sample ($M_{n,\text{GPC}}$ = 5700) obtained from GPC using polystyrene standards and the theoretical value (M_n = 4700) calculated using the equation 2. The molecular weight can be also calculated by using the both end groups, aromatic peaks "h" at 7.0−7.7 ppm, corresponding to 8 protons ($M_{n,\text{NMR}}$ = 6000 in the methylated form). These results suggest the formation of poly(A-Hyp-OH) with the CTA fragments at the polymer chain ends (α-chain end with the benzyl group and the ω-chain end with phenyldithioester). This spectroscopic method was also valid for the poly(A-Hyp-OH) obtained at $[\text{A-Hyp-OH}]_0:[\text{CTA 1}]_0$ ratio of 50 ($M_{n,\text{NMR}}$ = 9800 in the methylated form, $M_{n,\text{GPC}}$ = 10000, and $M_{n,\text{theory}}$ = 9700). In contrast, slightly higher molecular weight was calculated using the peak "h" attributed to the Z-fragment end group ($M_{n,\text{NMR}}$ = 15700 in the carboxylic acid form).

Chain extension experiment was also conducted using the dithiobenzoate-terminated poly(A-Hyp-OH) (M_n = 10000, M_w/M_n = 1.30, conversion = 89%), which was independently prepared by the polymerization at $[\text{A-Hyp-OH}]_0/[\text{CTA 1}]_0/[\text{AIBN}]_0$ = 100/2/1 in DMF at 60 °C for 24 h. For the synthesis of the chain extended polymer, the polymerization was also carried out in DMF at 60 °C with a molar ratio the macro-CTA/AIBN of 2 in order to achieve sufficient monomer conversion and to minimize any loss of the dithioester end group. Under the condition, the polymer yield was 64% after 48 h at the ratio $[\text{A-Hyp-OH}]_0/[\text{macro-CTA}]_0/[\text{AIBN}]_0$ = 300/2/1. As can be seen in Figure 7, a clear shift in the SEC trace toward higher molecular weight region is seen after the chain extension. These results suggest that the most of the chain end of poly(A-Hyp-OH) is capped with the fragments of CTA.

Thermal Phase Transition in Aqueous Solution. Figure 8 compares the thermally induced phase separation behaviors of poly(A-Pro-OMe) and poly(A-Hyp-OMe) prepared by RAFT polymerization of unprotected monomers (A-Pro-OH and A-Hyp-OH), followed by methylation, as monitored by UV (500 nm) in water. In the case of the poly(A-Pro-OMe) having narrow polydispersity, the transmittance decreased sharply in the solution at a specific temperature (17.5 °C) on heating, indicative of a sharp phase transition (LCST type). The behavior is almost the same to that of poly(A-Pro-OMe) prepared by RAFT polymerization of the methylated monomer, A-Pro-OMe.[58,59] In contrast, poly(A-Hyp-OMe), was soluble in water at low temperature, which undergo a clear phase transition upon heating

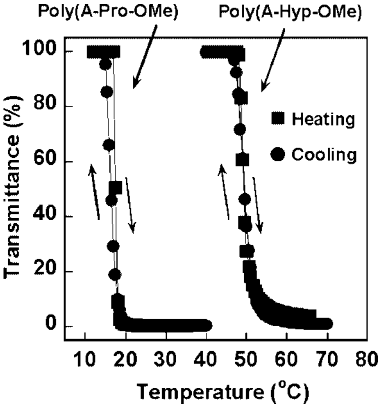


Figure 8. Temperature dependence of the transmittance at 500 nm of aqueous solutions (2.0 mg/mL) of poly(A-Pro-OMe) (M_n = 12200, M_w/M_n = 1.26) and poly(A-Hyp-OMe) (M_n = 11000, M_w/M_n = 1.29) prepared by RAFT polymerization of A-Pro-OH and A-Hyp-OH, respectively, followed by methylation.

Table 5. Summary of Polymerization Conditions and Properties of Random Copolymers Prepared by Copolymerization of *N*-Acryloyl-L-proline Methyl Ester (A-Pro-OMe) and *N*-Acryloyl-4-trans-hydroxy-L-proline (A-Hyp-OH) in the Presence of Chain Transfer Agent (CTA) at 60 °C for 24 h[a]

entry	feed ratio (A-Pro-OMe:A-Hyp-OH)	yield[b] %	M_n [c](M_w/M_n)	x:y[d]	LCST[e] °C
1[f]	100:0	90	12200(1.26)	100:0	17.5
2[f]	75:25	96	9600(1.20)	76:24	20.1
3[f]	50:50	96	8900(1.28)	60:40	24.0
4[f]	25:75	97	10000(1.44)	32:68	30.0
5[g]	0:100	88	11000(1.29)	0:100	49.5

[a] $([\text{A-Pro-OMe}]_0 + [\text{A-Hyp-OH}]_0)/[\text{CTA}]_0$ = 50, $[\text{CTA}]_0/[\text{AIBN}]_0$ = 2, monomer concentration = 0.25 g/mL, where AIBN = 2,2′-azobis(isobutyronitrile), A-Pro-OMe = *N*-acryloyl-L-proline methyl ester, A-Hyp-OH = *N*-acryloyl-4-*trans*-hydroxy-L-proline. [b] Isolated yield after precipitation with diethyl ether:acetone (7:3 vol %). [c] Methylated samples were measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr). [d] Comonomer composition (*x* = A-Pro-OMe, *y* = A-Hyp-OMe) determined by ^{1}H NMR in CD_3OD. [e] LCST (lower critical solution temperature) of the methylated samples estimated by UV spectroscopy. [f] Polymerization with benzyl 1-pyrrolecarbodithioate (CTA 2) in MeOH. [g] Polymerization with benzyl dithiobenzoate (CTA 1) in DMF.

at higher temperature (49.5 °C), suggesting that the addition of hydroxyl group in the monomer unit leads to a drastic increase in the transition temperature. The transmittance decreased sharply during 49−51 °C, indicating that the phase separation was highly sensitive. When the white turbid solution was cooled, it returned to the homogeneous state. In both cases, this phase separation was not only extremely sensitive but also reversible on heating and cooling without hysteresis.

In LCST-type phase separation, the occurrence and position of a phase transition temperature are known to depend on the distribution of hydrophilic and hydrophobic groups within the polymers. With this in mind, we attempted to manipulate LCST of the amino acid−based polymers by the copolymerization of A-Pro-OMe with a hydrophilic monomer, A-Hyp-OH, followed by the methylation, by which appropriate hydrophilic/hydrophobic balance can be achieved. The copolymerization via RAFT process was conducted at different A-Pro-OMe:A-Hyp-OH molar ratio in the feed at ([A-Pro-OMe]+[A-Hyp-OH])/[CTA] = 50 and [CTA 2]/[AIBN] = 2. The results are summarized in Table 5. Depending on the comonomer ratio (A-Pro-OMe:A-Hyp-OH = 75:25, 50:50, 25:75) in the feed, the copolymers with number-average molecular weights between 8900 and 10000 and the A-Hyp-OMe content between 24 and 68% were obtained quantitatively (yield >95%) at 60 °C after 24 h. The compositions of the copolymers determined by ^{1}H

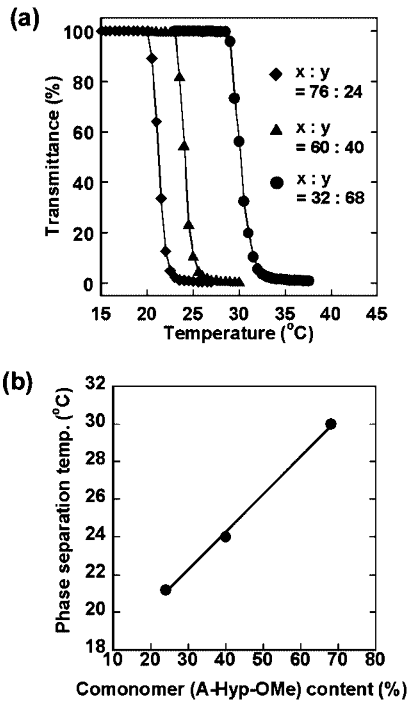


Figure 9. (a) Temperature dependence of the transmittance at 500 nm of aqueous solutions of the copolymers (2.0 mg/mL; x = A-Pro-OMe = 0.76, 0.60, and 0.32; y = A-Hyp-OMe), and (b) relationship between the phase separation temperature and the comonomer (A-Hyp-OMe) composition in the random copolymers.

NMR are in reasonable agreement with the calculated values from the conversion and the feed ratio of both monomers, irrespective of the comonomer ratio in the feed. The polydispersity indices (M_w/M_n) for all samples ranged between 1.20 and 1.44. Figure 9a shows the dependence of the solution turbidity of the copolymers having different A-Hyp-OMe contents on the temperature. The transmittance decreased sharply in all aqueous solutions at a specific temperature on heating, indicative of a sharp phase transition (LCST type). The mole fraction of A-Pro-OMe, x, was found to determine the temperature of the phase separation, varying from 17.5 °C at $x = 1.0$ to 30 °C at $x = 0.32$ for a 2.0 mg/mL aqueous solution. The phase separation occurred with a similar sensitivity in all cases, irrespective of the ratio of A-Hyp-OMe to A-Pro-OMe. The LCST increased linearly with increasing A-Hyp-OMe content (Figure 9b). It was demonstrated that the copolymerizations via RAFT afforded the amino acid−based polymers having well-defined structure, and the transition temperature can be manipulated in the range of 17−50 °C by the hydrophilic comonomer composition.

Conclusion

This work presented the controlled syntheses of weak polyelectrolyte, poly(A-Pro-OH), water-soluble polymer, poly(A-Hyp-OH), and thermoresponsive polymers, poly(A-Pro-OMe), poly(A-Hyp-OMe), and poly(A-Pro-OMe-*co*-A-Hyp-OMe)s, by RAFT polymerization of the acrylamides containing proline and hydroxyproline moiety. In addition to precise polymerization of A-Pro-OH having a carboxylic acid moiety, we have demonstrated successful controlled radical polymerization of A-Hyp-OH, in which a carboxylic acid and a hydroxyl group in the monomer unit are intact without protecting group, using suitable CTA and polymerization conditions. The methylation reaction of the carboxylic acid groups in poly(A-Pro-OH) and poly(A-Hyp-OH) led to thermoresponsive polymers having different phase separation temperatures, 17.5 °C for poly(A-Pro-OMe) and 49.5 °C for poly(A-Hyp-OH), respectively. The transition temperature of the random copolymers, poly(A-Pro-OMe-*co*-A-Hyp-OMe)s, could be manipulated by the monomer composition. We believe that this paper represents the first report on controlled synthesis of amino acid−based polymers having characteristic thermoresponsive property, in which the transition temperature could be manipulated by the composition of two chiral monomers. To conclude, RAFT polymerization proves to be an effective technique for the direct synthesis of well-defined amino acid−based polymers without protective group chemistry. We are currently extending this procedure to the design and synthesis of amino acid−based polymers having various architectures, such as graft, star, and block copolymers, with characteristic chiroptical and stimuli-responsive properties.

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Supporting Information Available: Figures showing ^{1}H and ^{13}C NMR and FT-IR spectra of the monomers, polymers, and a representative random copolymer, figures shwoing SEC traces of methylated poly(A-Pro-OH)s and poly(Hyp-OH)s obtained with different CTAs, and tables summarizing the data for effects of $[M]_0/[CTA]_0$. This material is available free of charge via the Internet at http://pubs.acs.org.

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