Controlled synthesis of thermoresponsive polymers derived from L-proline *via* RAFT polymerization†

Hideharu Mori,* Hideyuki Iwaya, Atsushi Nagai and Takeshi Endo*

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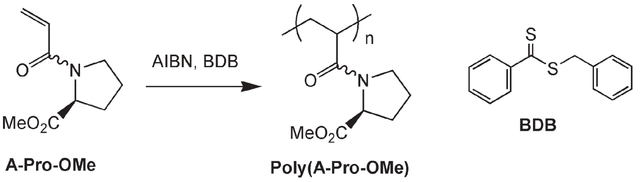
Well-defined polymers derived from L-proline are synthesized using reversible addition–fragmentation chain transfer (RAFT) polymerization and the amino acid-based polymers exhibit thermosensitive phase separation at lower critical solution temperatures (LCST = 15–45 °C) in aqueous medium.

Proteins can be regarded as the ultimate polymers for synthetic chemistry, as they possess high molecular weights with uniform chain lengths and precisely controlled monomer sequences, which lead to hierarchical folding and organization. Incorporation of amino acid residues, which are the constitutional components of proteins, into synthetic polymers is of interest, because such a combination may create new nonbiological macromolecules with biomimetic structures and properties. So-called "intelligent" or "smart" materials that can sense signals and produce a definite dynamic response in the form of a change in shape, size, or structure, are another feature central to developments in various scientific fields.[1] During recent years, there has been increasing attention paid to artificial and model proteins having stimuli-responsive properties, in which these repeating sequences hold and/or self-assemble into more highly ordered structures when the temperature is raised in water.[2,3] Polymers susceptible to reversible thermoprecipitation from aqueous solution have also been used as simple analogs to the solubility and denaturing of proteins and biopolymers.[4]

We present here the controlled synthesis of amino acid-based polymers derived from L-proline, which have characteristic thermoresponsive properties in aqueous medium (Scheme 1). L-Proline is one of the most important amino acids, as it is responsible for various functions and ordered structures, such as turn inducement in natural peptides and proteins,[5,6] and the helical structure of oligoprolines.[7,8] The attractive features of L-proline have promoted a number of studies of derivatives,[9,10] polymeric materials,[11] and their bio-related phenomena and applications.[12,13] In this study, we focused on poly(*N*-acryloyl-L-proline methyl ester), poly(A-Pro-OMe), which is known to exhibit a characteristic LCST (lower critical solution temperature) at 14 °C in aqueous solution.[14,15] However, the preparation of the thermosensitive polymer is based entirely on radiation-induced polymerization[14,15] or conventional free radical polymerization,[11] which yields poorly defined polymers. For the precise manipulation of the thermosensitive property and understanding the folding and assembly of protein constructs, it is important to produce polymers possessing narrow chain length distributions, which can assemble linear macromolecules into precisely defined nanostructures. Furthermore, reversibility and transition sharpness of stimuli-responsive systems can be achieved by utilizing polymers with well-defined primary structures, attainable *via* controlled/living polymerization.[16,17]



Scheme 1 RAFT polymerization of A-Pro-OMe in the presence of BDB as a chain transfer agent.

To provide an effective route for the controlled synthesis of amino acid-based polymers derived from L-proline, we employed reversible addition–fragmentation chain transfer (RAFT) polymerization[18,19] One of the key points for the synthesis of well-defined products *via* the RAFT process is the design of the chain transfer agent (CTA), as the choice of R and Z groups depends on the monomer.[20,21] From our preliminary results using various CTAs, benzyl dithiobenzoate (BDB) was found to be efficient as the CTA for the preparation of near-monodisperse poly(A-Pro-OMe)s with controlled molecular weights (see the Supplementary Information, Table S1†). When A-Pro-OMe was polymerized using BDB as CTA with AIBN as an initiator at $[\text{A-Pro-OMe}]_0 : [\text{CTA}]_0 : [\text{AIBN}]_0 = 100 : 2 : 1$ in chlorobenzene, full conversion was obtained at 60 °C after 20 h, and the resulting polymer showed a sharp symmetrical SEC peak ($M_w/M_n = 1.15$) without shoulder and tailing. During the polymerization, the characteristic pale red solution stayed constant without significant change in the color and viscosity. The number-average molecular weight of the polymer, measured by a GPC in DMF with 10 mM LiBr, was $M_n = 5500$, which is comparable to the theoretical value ($M_n = 7000$) calculated from the monomer : CTA molar ratio and the monomer conversion.

The controlled character of the RAFT polymerization of A-Pro-OMe was investigated in terms of the time-dependent change in the molecular weights and polydispersity of the resulting polymers. When the polymerization was conducted in chlorobenzene at 60 °C at a constant chain transfer agent : initiator molar

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Department of Polymer Science and Engineering, Faculty of Engineering, Yamagata University, 4-3-16, Jonan, Yonezawa, 992-8510, Japan. E-mail: h.mori@yz.yamagata-u.ac.jp; tendo@yz.yamagata-u.ac.jp; Fax: +81-238-26-3092; Tel: +81-238-26-3765

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ratio, $[BDB]_0 : [AIBN]_0 = 2 : 1$, more than 90% conversion was reached within 5 h (see the Supplementary Information, Figure S1†). The SEC traces of poly(A-Pro-OMe) at different polymerization times clearly illustrate the increase in molar mass with time, as shown in Fig. 1b, and symmetrical unimodal SEC peaks ($M_w/M_n = 1.12$–1.18) without shoulders and tailings are observed for the polymers obtained even at higher conversion (>90%). The linearity of the M_n *versus* conversion plot (Fig. 1a) clearly indicates that the polymerization proceeded in a controlled fashion with a constant number of propagating chains throughout the polymerization.

In the next stage, the polymerization was conducted at different monomer : chain transfer agent ($[\text{A-Pro-OMe}]_0 : [BDB]_0$) ratios between 25 and 150 in chlorobenzene at 60 °C for 20 h, and the AIBN : BDB molar ratio was held constant at 1 : 2. Under the conditions, the conversions were quantitative (>99%, as determined by ^{1}H NMR) in all cases. Fig. 2 shows the relation of the molecular weight and polydispersity with the $[\text{Monomer}]_0 : [\text{CTA}]_0$ ratio for the polymerization. A linear increase in the number-average molecular weight with the $[\text{A-Pro-OMe}]_0 : [BDB]_0$ ratio is observed clearly, and the molecular weight distribution remains narrow ($M_w/M_n = 1.13$–1.22). The feasibility to control the molecular weight based on the monomer : CTA molar ratio was confirmed, indicating good control of the polymerization. The resulting homopolymer, poly(A-Pro-OMe), was soluble in water at low temperature, and undergoes a clear phase transition upon heating at LCST around 15–20 °C, depending on the tacticity.

In this study, our attention was focused on the synthesis of amino acid-based polymers having not only well-defined architectures but also characteristic stimuli-responsive properties. In LCST-type phase separation, the occurrence and the position of a phase transition temperature are known to depend on the distribution of hydrophilic and hydrophobic groups within the polymers.[22] 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, *N*,*N*-dimethylacrylamide (DMA), by which an appropriate hydrophilic–hydrophobic balance can be achieved. Copolymerization *via* the RAFT process was conducted at different A-Pro-OMe : DMA molar ratios in the feed at $([\text{A-Pro-OMe}] + [\text{DMA}])/[BDB] = 50$ and $[BDB]/[AIBN] = 2$. The results are summarized in Table 1. Depending on the comonomer ratio (A-Pro-OMe : DMA = 95 : 5, 90 : 10, 80 : 20, 70 : 30, 50 : 50) in the feed, the copolymers with number-average molecular weights between 8300 and 5100 and an A-Pro-OMe content between 92 and 48% were obtained quantitatively (yield > 87%) at 60 °C after 20 h. The compositions of the copolymers determined by ^{1}H NMR are in good 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 1.22 and 1.28.

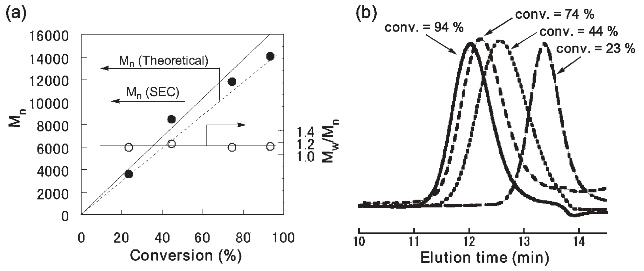

Fig. 1 (a) Number-average molecular weight and polydispersity as a function of conversion, and (b) evolution of SEC traces with conversion. Conditions: [A-Pro-OMe] = 1.0 M. $[\text{A-Pro-OMe}]_0/[BDB]_0 = 112.4$, $[BDB]_0/[AIBN]_0 = 2$, 60 °C.

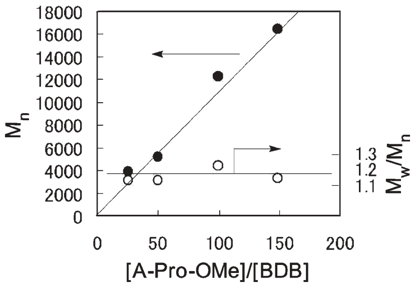

Fig. 2 (a) Dependence of the number-average molecular weight and molecular weight distribution on the $[\text{A-Pro-OMe}]_0 : [BDB]_0$ ratio for the polymerization.

Fig. 3a shows the dependence of the solution turbidity of the copolymers having different DMA 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,

Table 1 RAFT copolymerization of A-Pro-OMe with DMA[a]

Feed ratio (A-Pro-OMe : DMA)	Yield[b](%)	M_n[c](M_w/M_n)	*x* : *y*[d]	LCST[e]/°C
100 : 0	84	5500 (1.15)	100 : 0	15.0
95 : 5	99	8300 (1.25)	92 : 8	24.5
90 : 10	96	6800 (1.22)	83 : 17	30.5
80 : 20	87	6200 (1.25)	77 : 23	34.5
70 : 30	99	7000 (1.28)	67 : 33	43.5
50 : 50	95	5100 (1.24)	48 : 52	—

[a] Conditions: $([\text{A-Pro-OMe}] + [\text{DMA}])/[BDB] = 50$, $[BDB]/[AIBN] = 2$, 60 °C, 20 h. [b] Isolated yield after precipitation with diethyl ether. [c] Estimated by SEC [DMF, LiBr (10 mM)]. [d] Comonomer composition (*x* = A-Pro-OMe, *y* = DMA) determined by ^{1}H NMR spectroscopy. [e] Estimated by UV spectroscopy ($c = 1$ g L^{-1})

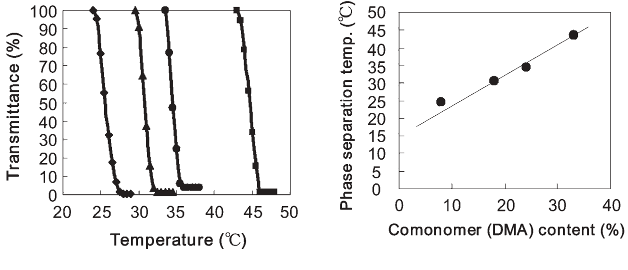

Fig. 3 (a) Temperature dependence of the transmittance at 500 nm of aqueous solutions of the copolymers (1.0 mg mL^{-1}; *x* = A-Pro-OMe = 0.92, 0.83, 0.77, and 0.67; *y* = DMA), and (b) the relationship between the phase separation temperature and the comonomer (DMA) composition in the copolymers.

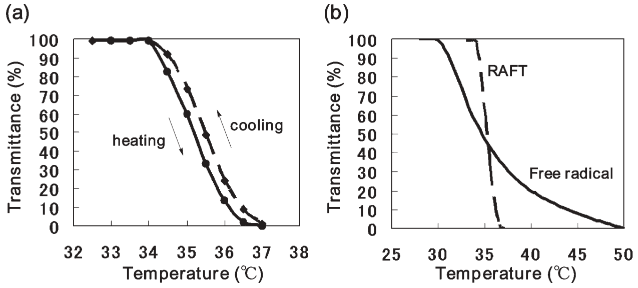

Fig. 4 Temperature dependence of the transmittance at 500 nm in aqueous solution (1.0 mg mL^{-1}). (a) Heating–cooling cycle of the copolymer solution obtained by RAFT (M_n = 6200, M_w/M_n = 1.25, x = A-Pro-OMe = 0.77, y = DMA = 0.23), and (b) comparison of the copolymers obtained by RAFT and conventional free radical polymerization (M_n = 242 000, M_w/M_n = 2.25, x = 0.68, y = 0.32).

varying from 15 °C at x = 1.0 to 44 °C at x = 0.67 for a 1.0 mg mL^{-1} aqueous solution. The phase separation occurred with a similar sensitivity in all cases, irrespective of the ratio of DMA to A-Pro-OMe. The LCST increased linearly with increasing DMA content (Fig. 3b), whereas the LCST phenomenon disappeared when hydrophilic DMA comprises more than 50% of the copolymers. It was demonstrated that the copolymerizations *via* RAFT afforded amino acid-based polymers having well-defined structures, and the transition temperature can be manipulated in the range 15–45 °C by the hydrophilic comonomer composition.

In the case of the copolymer containing 23% DMA, the aqueous solution transformed into an opaque liquid when warmed to above 33 °C, as monitored by the transmittance at 500 nm. The transmittance decreased sharply between 33.5 and 36.5 °C, as can be seen in Fig. 4a, indicating that the phase separation was highly sensitive. When the white turbid solution was cooled, it returned to the homogeneous state. Note that this phase separation was not only extremely sensitive but also reversible on heating and cooling without hysteresis. Fig. 4b compares the temperature-dependent solution turbidity of the copolymers obtained by the RAFT process and conventional free radical copolymerization of A-Pro-OMe and DMA under similar conditions. The transition curve of the copolymer obtained by free radical polymerization becomes broad, and the transition width is approximately 20 °C (30–50 °C) on heating. The significant difference in the transition behavior between the copolymers suggests that reversibility and transition sharpness of the thermoresponsive system can be achieved by utilizing the amino acid-based copolymers having well-defined primary structures.

In conclusion, we have demonstrated that the RAFT polymerization of an acrylamide having the L-proline moiety allows the synthesis of amino acid-based polymers with pre-determined molecular weights, a narrow molecular weight distribution, and characteristic thermosensitive properties in aqueous medium. To the best of our knowledge, this is the first example of the synthesis of well-defined thermoresponsive polymers derived from an amino acid, in which sensitive phase separation behavior in water can be tuned simply by choosing an appropriate hydrophilic–hydrophobic balance. We are currently extending this procedure to the design and synthesis of highly ordered hierarchical structures from amphiphilic block copolymers and intelligent hybrid materials derived from L-proline. The self-organization of the well-defined amino acid-based polymers can provide a viable route to the production of tailored materials with unique intelligent properties for various biological and medicinal applications, such as controlled release, biochemical sensing, and biocompatible materials.

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