Synthesis and characterization of new temperature-responsive polymers, poly(*N*-acryloylpiperidine) derivatives

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Abstract

Poly(N-acryloyl-nipecotamide) (PNANAm), poly(N-acryloyl-isonipecotamide) (PNAiNAm), and poly(N-acryloyl-N,N-diethylnipecotamide) (PNADNAm) were synthesized as novel temperature-responsive polymers using reversible addition-fragmentation chain-transfer polymerization. Aqueous solutions of these three polymers were examined via temperature-dependent optical transmittance measurements. The PNANAm sample with a hydrophilic terminal group showed an upper critical solution temperature (UCST) in phosphate-buffered saline (PBS) when its molecular weight (Mn) was 7,600 or higher, whereas PNANAm (Mn < 7,600) was soluble. The UCST was influenced by molecular weight and the polymer concentration. In contrast, PNANAm sample with nonionic terminal group showed UCST, when Mn was below 7,600, suggesting that the terminal nonionic group possibly increased UCST of PNANAm. The urea addition experiment suggested that the driving force for expression of UCST of PNANAm is the formation of inter-and intramolecular hydrogen bonds among the polymer chains. PNAiNAm was soluble in PBS but exhibited an UCST in an appropriate concentration of ammonium sulfate. In contrast, PNADNAm exhibited a lower critical solution temperature. Comparing the chemical structure of these polymers and their phase transition behaviors suggests that the carboxamide group position in the piperidine ring could determine the UCST expression. These results could help design temperature-responsive polymers with a desired the cloud point temperature.

Introduction

The author previously developed new temperature-responsive polymers that exhibit LCST behavior and demonstrated that these polymers could interact in a more hydrophobically manner with proteins and peptides compared with conventional temperature-responsive PIPAAm under physiological conditions.[1] In the course of the development of those polymers, it was found that poly(*N*-acryloyl-isonipecotamide) (PNAiNAm) (Fig. 1), which contains a carboxamide group at position 4 of the piperidine ring of poly(*N*-acryloyl-piperidine) (PAP), exhibits cloud point temperature like UCST in aqueous solutions (Figure 2).[2] This finding gave the author anticipation that poly(N-acryloyl-nipecotamide) (PNANAm) (Fig. 1), which is a derivative of PNAiNAm and contains a carboxamide group at position 3 of the piperidine ring of PAP, would also show a UCST in aqueous solutions (Fig. 2). Although, based on the anticipation, PNANAm was prepared using the conventional radical polymerization method and showed cloud point temperature like UCST, their temperature-dependent optical transmittance curves were broad in aqueous conditions over the entire range of investigated temperatures (Fig. 2). This was due to a wide range of molecular weights of the PNANAm. By contrast, living radical polymerization techniques, such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain-transfer (RAFT) polymerization, have been exploited for the synthesis of UCST-type polymers with controlled molecular weights and narrow molecular weight distributions.[3]

In this study, in order to prepare PNANAm with precisely controlled molecular weight and narrower molecular weight distribution and investigate the cloud point of PNANAm as UCST-type temperature-responsive polymer, RAFT polymerization method was utilized using two different chain transfer agents (Scheme 1). In addition, besides PNANAm, PAP derivatives such as PNAiNAm and *N*-acryloyl-*N,N*-diethylnipecotamide (NADNAm) were prepared and investigated in the same way.

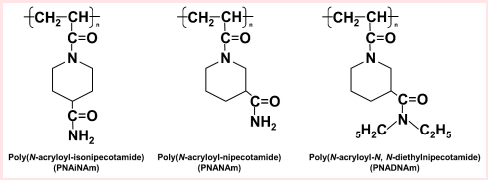


Fig. 1. Chemical structures of poly(*N*-acryloyl-isonipecotamide) (PNAiNAm), poly(*N*-acryloyl-nipecotamide) (PNANAm) and poly(*N*-acryloyl-*N,N*-diethylnipecotamide) (PNADNAm)

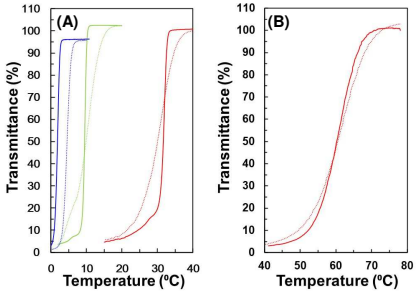


Fig. 2. Temperature responsive polymers, (A) PNAiNAm and (B) PNANAm prepared by the conventional free radical polymerization method. Temperature dependent optical transmittance for (A) PNAiNAm (1.0 wt%) dissolved in PBS in the presence of ammonium sulfate (500mM (red), 300mM (green), and 200mM (blue)) and (B) PNANAm (0.8wt%) (red) in PBS. Solid and dotted lines indicates cooling and heating processes, respectively.

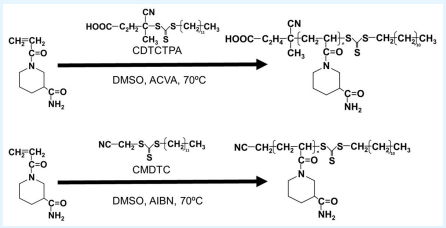
Experimental

Monomer synthesis: Nipecotamide (5.0 g) was dissolved in DMF (80 mL) containing trimethylamine (TEA) (7.1 mL) in an ice-cooled bath. Acryloyl chloride (4.7 mL) dissolved in DMF (20 mL) was dripped into the cooled solution in a N_2 atmosphere. After the addition of the acryloyl chloride solution, the reaction solution was stirred for 5 h at room temperature. Next, the precipitate was removed via filtration, and the filtrate was concentrated using a rotary evaporator. The concentrated crude product was purified by column chromatography performed on silica gel using an ethyl acetate/acetone mixture (70:30 (v/v)) as the eluent. The eluted solution with the desired monomer component was collected, concentrated, and recrystallized by leaving the solution in an ice-cooled bath for a few hours. The recrystallized monomer was filtered and dried under reduced pressure at room temperature for 10 h (yield 72%). Other monomers such as NAiNAm and NADNAm were also synthesized in the same way. Detailed procedure was indicated in a previous report.[4]

Synthesis of PNANAm by RAFT polymerization: NANAm (1.0 g), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]-pentanoic acid (CDCTPA; 11.0 mg), and 4,4′-azobis(4-cyanovaleric acid) (ACVA; 1.54 mg) were dissolved in DMSO (10 mL). CDCTPA and ACVA were used as chain transfer agent (CTA) and initiator, respectively. The mixture was degassed by subjecting it to three freeze–thaw cycles, and then it was sealed under reduced pressure. Polymerization was performed at 70ºC for 24 h (Scheme 1). After polymerization, the reaction solution was dripped onto an acetone/methanol mixture (70:30 (v/v)) to precipitate the polymer. The polymer was then filtered and dried under vacuum at room temperature (yield 84%) (Table 1, Run 1). Similarly, PNANAm samples with different molecular weights and terminal groups were synthesized using different concentrations of the CTA and/or different CTAs such as 4-cyano-4-(((dodecylthio)carbonothioyl)thio)pentanoic acid (CMDTC). 2, 2-azobisisobutyronitrile (AIBN) was used as the initiator when cyanomethyl dodecyl trithiocarbonate (CMDTC) was added to the reaction solution as the CTA. The reaction conditions are listed in Table 1. PNAiNAm and PNADNAm were also prepared in the same way (Table 2).

Results and Discussion

Scheme 1 shows the synthesis of PNANAm using the RAFT polymerization method. The polymerization conditions and properties of the synthesized PNANAm samples are listed in Runs 1–10 in Table 1. Both terminal groups of the PNANAm chain depend on the chain transfer agent (CTA), such as 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]-pentanoic acid (CDCTPA) and cyanomethyl dodecyl trithiocarbonate (CMDTC). Both CTAs were used to provid a terminal group with a hydrophobic dodecyl group to one side of the PNANAm chains. Another terminal group was the 4-cyano-pentanoic acid group (Runs 1–5) or nonionic cyano-ethyl group (Runs 6–10), depending on the CTA species used, as indicated in Scheme 1



Scheme 1. Synthesis of PNANAm with (A) 4-cyano-pentanoic acid group and (B) nonionic cyano-ethyl group as terminal groups using RAFT polymerization method

Table 1 Polymerization conditions and properties of PNANAm

	Molar ratio						
Code	Monomer	CTA	Initiator	Time (h)	Yield (%)	Mn	Mw/Mn	in PBS
Run 1	1000	5[a]	1[c]	24	84.0	10700	1.37	UCST[a]
Run 2	670	5[a]	1[c]	24	93.9	7600	1.33	UCST[f]
Run 3	500	5[a]	1[c]	24	98.3	6300	1.38	Soluble
Run 4	330	5[a]	1[c]	24	96.0	4000	1.42	Soluble
Run 5	200	5[a]	1[c]	24	86.2	2800	1.30	Soluble
Run 6	1000	5[b]	1[d]	4	92.2	11300	1.48	UCST[g]
Run 7	850	5[b]	1[d]	4	97.1	8600	1.50	UCST[g]
Run 8	700	5[b]	1[d]	3	92.7	8300	1.38	UCST[h]
Run 9	400	5[b]	1[d]	1	86.4	4600	1.41	UCST[g]
Run 10	200	5[b]	1[d]	24	88.3	2700	1.35	UCST[i]

a) 4-Cyano-4-[(dodecylsulfanyl-thiocarbonyl)sulfanyl]-pentanoic acid (CDCTPA), b) Cyanomethyl dodecyl trithiocarbonate (CMDTC), c) 4,4′-azobis(4-cyanovaleric acid) (ACVA), d) 2,2′-azodiiso-butyronitrile (AIBN), e) 0.1-0.6 wt%, f) 0.5-8.0 wt%, g) 0.1-0.3 wt%, h) 0.1 wt%, i) 0.5-6.0 wt%

The molecular weight (Mn) of PNANAm was modulated from 2,700 to 11,300 by using different initial monomer/CTA molar ratios. The molecular weight distribution (Mw/Mn) of PNANAm was found to range between 1.30 and 1.50. These results suggested that the molecular weight of the PNANAms was controlled by the RAFT polymerization method. Terminal group species as expected would also be introduced, dependent on the initiator and CTA species (Scheme 1). Regarding the PNANAm with the hydrophilic terminal group (Runs 1–5), the PNANAm samples whose Mn was lower than 7,600 (Runs 3–5) were readily soluble in PBS solution and did not show the cloud point temperature.

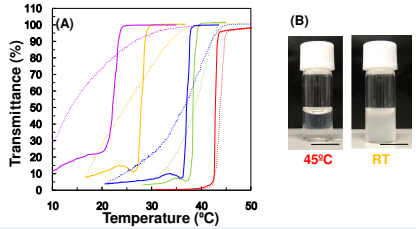


Fig. 3. Temperature-dependent optical transmittance of PNANAm (Run 2) solutions (PBS). PNANAm concentration was (A) 4.0 wt% (red), 2.0 wt% (green), 1.5 wt% (blue), 1.0 wt% (yellow), and 0.5 wt% (purple); (B) 4.0 wt% (red), 6.0 wt% (gray), and 8.0 wt% (black) of PNANAm solution. Solid and dotted lines indicate cooling and heating processes, respectively. (B) Photographs of PNANAm solutions (PBS) (Run 2, 2.0 wt%) at (left) 45ºC (above UCST) and (right) room temperature (below UCST). Scale bar = 1.0 cm.

The temperature-dependent optical transmittance curves of PNANAm (Run 2) dissolved in PBS solution in various concentrations are given in Fig. 3(A). It is evident that PNANAm exhibited a temperature-change-induced cloud point temperature. The PNANAm was soluble at higher temperatures but underwent aggregation at temperatures lower than the cloud point temperature (Fig. 3(B)). However, in the presence of urea (2 M), which is utilized as a hydrogen-bond-breaking agent,[32] the cloud point temperature was disappeared. The PNANAm solution containing urea was transparent irrespective of temperature. A similar tendency was also observed in other PNANAm samples (Runs 1 and 6–10). This result indicated that the appearance of the cloud point temperature of PNANAm is mediated by the formation of inter- and intramolecular hydrogen bonds between the PNANAm chains via the carboxamide group, as described below. Thus, it can be concluded that PNANAm is a novel temperature-responsive polymer that exhibits a cloud point temperature, UCST. The UCST decreased with a decrease in the concentration of the PNANAm solution to 4.0 wt% (42.8ºC) and lower (0.5 wt%, 23.3ºC) (Fig. 3).

Table 2 Polymerization conditions and molecular weights of PNAiNAm and PNADNAm

	Molar ratio						
Code	Monomer	CTA	Initiator	Time (h)	Yield (%)	Mn	*Mw/Mn*
PNAiNAm	200	5	1	24	94.0	3800	1.32
PADNAm	200	5	1	24	95.1	3300	1.39

NAiNAm and NADNAm were also synthesized as analogs and derivatives of NANAm for the synthesis of PNAiNAm and PNADNAm (Fig. 4). The RAFT polymerization conditions for NAiNAm and NADNAm are summarized in Table 2. They have a hydrophobic dodecyl group and nonionic cyano-ethyl group at the terminal of polymer. The molecular weight distribution was relatively narrow. The temperature-dependent optical transmittances of the PNAiNAm and PNADNAm solutions were examined (Fig. 5). The PNAiNAm solution was transparent at all temperatures (Fig. 5(A), yellow). However, the PNAiNAm solution exhibited UCST characteristics in the presence of an appropriate amount of ammonium sulfate (Fig. 5(A)). The appearance of the UCST behavior can be attributed to the sulfate ions, which decreased the solubility of PNAiNAm as a kosmotropic agent. Conversely, PNADNAm bearing a *N,N*-diethyl carboxamide group at position 3 of the piperidine ring of PNANAm exhibited a LCST in PBS solution (Fig. 5(B)). The LCST of PNADNAm increased with a decrease in the polymer concentration.

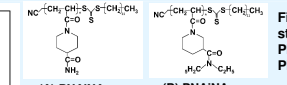


Fig. 4. Chemical structure of (A) PNAiNAm and (B) PNADNAm

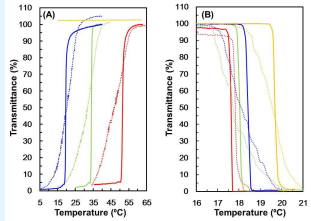


Fig. 5. Temperature-dependent optical transmittance of (A) PNAiNAm and (B) PNADNAm solutions (PBS). In the presence of (A) 600 mM (red), 500 mM (green), and 400 mM ammonium sulfate (blue) and without ammonium sulfate (yellow). PNADNAm concertation of (B) 3.0 wt% (red), 1.0 wt% (green), 0.5 wt% (blue), and 0.1 wt% (red). Solid and dotted lines indicate cooling and heating processes, respectively.

Conclusion

The temperature-dependent optical transmittance of resultant PNANAm and PNAiNAm solution employing appropriate conditions exhibited characteristics of UCST. It has been reported that polymers containing amide, carboxamide, and ureido groups, which act as proton donors or acceptors or both, are likely to exhibit UCST characteristics. Results of this study indicated that carboxamide group of PNANAm and PNAiNAm would provide proton donor and acceptor sites, enabling to express UCST character via temperature-dependent hydrogen-bonding formation based inter- and intramolecular interaction. In contrast, PNADNAm, which was bearing diethyl carboxamide groups instead of such proton donor and acceptor sites as carboxamide group, showed LCST character. Resultant UCST and LCST of PNANAm, PNAiNAm, and PNADNAm could be tuned in physiological conditions by using appropriate molecular weight, polymer concentration, and terminal group species.

To the best of my best knowledge, there has been no report on how the position of those functional groups in the side chains of polymers affects the resulting temperature-responsive behavior. This information, namely, the position and species of the functional groups in the polymer side chain, can provide valuable insights into the design and synthesis of novel temperature-responsive polymers that show a UCST or LCST under physiological conditions.

References. [1] Y. Akiyama, Y. Shinohara, Y. Hasegawa, A. Kikuchi, T. Okano, J. Polym. Sci., Part A: Polym. Chem. 2008, 46, 5471., [2] Y. Akiyama, K. Yoshizako, Y. Hasegawa, T. Okano, US patent 6,956,077 B1 2005., [3] A. Fujihara, N. Shimada, A. Maruyama, K. Ishihara, K. Nakai, S.-i. Yusa, Soft Matter 2015, 11, 5204., S. Glatzel, N. Badi, M. Päch, A. Laschewsky, J.-F. Lutz, Chem. Commun. 2010, 46, 4517., B. A. Pineda-Contreras, F. Liu, S. Agarwal, J. Polym. Sci., Part A: Polym. Chem. 2014, 52, 1878., [4] Y. Akiyama., Macromol Rapid Commun., 2021, 42, 2100208