Facile Synthesis of Temperature- and pH-responsive Dendritic-Linear-Dendritic Copolymer

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Dendritic–linear–dendritic triblock copolymers have attracted much attention due to their unique topology and special features. However, facile methods are still necessary to synthesize uniform dendritic–linear–dendritic copolymers. Here, we report an effective strategy for the synthesis of a temperature- and pHresponsive dendritic–linear–dendritic poly(amido amine)-poly(*N*isopropylacrylamide)-poly(amido amine) (PAMAM-PNIPAM-PAMAM), and evaluate the temperature- and pH-responsive properties of PAMAM-PNIPAM-PAMAM.

Keywords: Dendrimer | Dendritic-linear-dendritic copolymers | Temperature and pH responsivity

Discovering novel and facile methods to prepare polymers with different topologies has become one of the focus areas of polymer chemistry due to the fact that topology has a significant influence on polymer properties.¹ Owing to their compact structure and numerous functional groups, dendrimers have wide potential applications in many fields.^{2–4} Various dendrimer-like polymers have been synthesized to mimic dendrimers.^{5–8} Compared with dendrimers, dendritic–linear–dendritic copolymers have some special properties because the linear block can endow them with some unique features, leading to numerous applications in gene and drug delivery.^{9–12}

Based on these reported dendritic-linear-dendritic copolymers, synthetic strategies can be generally divided into three categories. (1) Coupling method, linear polymer chain reacts with a reactive dendron, which requires strict reaction conditions, and the uniform dendritic-linear-dendritic copolymer cannot be obtained because some unreacted dendrons remain in the product.¹³⁻¹⁵ (2) Macro reagent method, two dendrons are connected by a small molecular initiator or chain transfer reagent to form macro reagents, and the macro reagents were used in controlled radical polymerizations to form dendritic-lineardendritic copolymers. However, the formation of a uniform macro reagent is very difficult because it is very hard to remove unreacted dendrons.^{16,17} (3) Divergent method, the linear polymer block with dendritic blocks at both ends is prepared through iterative multistep reactions.^{9–12,18–22} The popularity of the divergent method is limited due to the lack of a convenient way to introduce more functional polymers as the linear block. Here, we combined reversible addition-fragmentation chain transfer (RAFT) polymerization and the divergent method for the synthesis of temperature- and pH-responsive dendriticlinear-dendritic PAMAM-PNIPAM-PAMAM, as shown in Figure 1. 1,2-Phenylenebis(methylene) didodecyl dicarbonotrithioate with two trithiocarbonate units was first used as the RAFT agent in the polymerization of N-isopropylacrylamide (NIPAM), producing poly(N-isopropylacrylamide) (PNIPAM) with two trithiocarbonate units at both ends. Subsequently, treating the PNIPAM with two trithiocarbonate ends using *n*-butylamine



Figure 1. Synthetic route of PAMAM-PNIPAM-PAMAM G 5.0.

formed PNIPAM with two thiol ends (HS-PNIPAM-SH).^{23,24} Then, the thiol was ready to react with methyl acrylate in very high efficiency via the Michael addition reaction,^{25,26} forming PNIPAM with two methyl ester ends (MA-PNIPAM-MA). Amine-capped PNIPAM (NH₂-PNIPAM-NH₂) was obtained by the amidation of MA-PNIPAM-MA with ethanediamine. At last, PAMAM-PNIPAM-PAMAM G 5.0 was synthesized using NH₂-PNIPAM-NH₂ as the linear block through iterative multistep reactions of Michael addition with methyl acrylate followed by amidation with ethanediamine. Using this method, well-defined dendritic–linear–dendritic polymers can be easily synthesized. Because each step has very high efficiency, the resulting PAMAM-PNIPAM-PAMAM is uniform.

RAFT polymerization of NIPAM using 1,2-phenylenebis(methylene) didodecyl dicarbonotrithioate as the chain transfer agent was carried out at 70 °C, producing PNIPAM with two trithiocarbonate ends (Figure 2A). Two PNIPAMs were synthesized, and the degrees of polymerization (DPs) of the PNIPAMs were 60 and 90 respectively, which were calculated based on ¹H NMR (Figure 2B) by comparing the protons integral values of the CH₂ neighbored S atom at 3.4 ppm to the pendent methine of the PNIPAM at 3.9–4.1 ppm. According to the GPC curves shown in Figure 3, the number-average molecular weight values (7900 and 11900) were in agreement with the DPs based on ¹H NMR analyses, and the polydispersity indexes (PDIs) of the



Figure 2. ¹H NMR spectra of 1,2-phenylenebis(methylene) didodecyl dicarbonotrithioate (A), PNIPAM₆₀ (B), MA-PNIPAM₆₀-MA (C), and NH₂-PNIPAM₆₀-NH₂ (D) in CDCl₃.



Figure 3. GPC curves of PNIPAM₆₀ and MA-PNIPAM₆₀-MA (A), PNIPAM₉₀ and MA-PNIPAM₉₀-MA (B) in DMF.

PNIPAMs were 1.16, suggesting that the polymerization of NIPAM was well-controlled.

The trithiocarbonate ends can be easily converted into thiol ends by the aminolysis of the trithiocarbonate units using *n*butylamine, as shown in Figure 1. Subsequently, the thiols reacted with methyl acrylate affords the desired MA-PNIPAM-MA.^{27,28} Because the aminolysis of the trithiocarbonate functions and the thiol-Michael addition reaction were both much faster than the addition of *n*-butylamine to methyl acrylate, these two steps can be conducted via a one-pot protocol. In order to avoid the oxidation of thiols by oxygen, the reaction mixture was stirred under an argon atmosphere. The thiol-Michael addition reaction of thiol with methyl acrylate was at such a high rate that the oxidative coupling reaction of thiols may have been suppressed. According to the GPC curves of the MA-PNIPAM-MA shown in Figure 3, no shoulder peak appeared at the higher molecular weight position, indicating that there was no combination of two thiols via oxidate coupling. In its ¹HNMR spectrum shown in Figure 2C, it is clear that the peak corresponding to dodecyl units disappeared while a new peak corresponding to methyl ester appeared, suggesting that methyl acrylate terminals have been linked onto the PNIPAM. The conversion of the thiol-Michael addition reaction, calculated by comparing the protons integral values of the CH₃ unit of methyl acrylate to the pendent methine proton of NIPAM at 3.9-4.1 ppm, was ca. 100%, indicating that almost all of the thiols had reacted with methyl acrylate. Subsequently, NH2-PNIPAM-NH2 was obtained by the amidation of MA-PNIPAM-MA with ethanediamine. In the ¹HNMR spectrum, the peak of the CH₃ unit corresponding to the methyl ester at 3.7 ppm disappeared (Figure 2D), suggesting that all of the methyl ester groups had reacted with ethanediamine. With these two reactions in one-pot, all of the PNIPAM with two trithiocarbonate ends had been converted into NH₂-PNIPAM-NH₂.

The dendritic-linear-dendritic PAMAM-PNIPAM-PAMAM was synthesized using NH₂-PNIPAM-NH₂ as the linear block. This route consists of two steps alternately repeated to achieve higher generations as follows the divergent synthesis technique. In the first step, Michael addition of methyl acrylate with the primary amine terminal groups results in a tertiary amine branch point with a methyl ester terminal. In the second step, the methyl ester terminal group undergoes amidation by ethanediamine to regenerate the primary terminal groups. Each step was monitored by ¹H NMR and GPC. We measured the molecular weights of PAMAM-PNIPAM-PAMAM G 3.5 and G 4.5 instead of G 4.0 and G 5.0 because PAMAM-PNIPAM-PAMAM G 4.0 and G 5.0 were insoluble in DMF. According to the GPC curves shown in Figure S12, no new peak appeared and all the PDIs of the PAMAM-PNIPAM-PAMAM polymers were lower than 1.22, indicating that all of the products were uniform dendritic-lineardendritic polymers.

In this study, the temperature-responsive behavior of PAMAM-PNIPAM-PAMAM G 5.0 was determined from the temperature dependence of the scattering intensity measured at a 90° scattering angle. This approach permits sensitive determination of the transition temperature even in polymer solutions of low concentrations exhibiting low levels of molecular association, which may be difficult to capture with the traditional method.²⁹ However, the tertiary amines inside the dendron were not protonated until the pH dropped to 5.2,30 Measurements about the pH-responsive behaviors of PAMAM-PNIPAM-PAMAM G 5.0 aqueous solution (1 mg mL^{-1}) were conducted at 25 °C, as shown in Figure 4. The tertiary amines inside the dendrons became nonprotonated when the pH of the solution increased to 8 from 5, and PAMAM dendrons became hydrophobic; therefore, PAMAM dendrons aggregated into nanoparticles with PNIPAM stabilizing the particles. We also investigated their temperatureresponsive behaviors at pH 5 and 8, as shown in Figure 5. At pH 5, the scattering intensity increased with the increase in temperature, which resulted from that PNIPAM became hydrophobic and aggregated, the aggregated nanoparticles were stabilized by PAMAM dendrons. At pH 8, the tertiary amines



Figure 4. The effect of pH on hydrodynamic diameter of PAMAM-PNIPAM₆₀-PAMAM G 5.0 (A) and PAMAM-PNIPAM₉₀-PAMAM G 5.0 (B). The variation of scattered light intensity of PAMAM-PNIPAM₆₀-PAMAM G 5.0 (C) and PAMAM-PNIPAM₉₀-PAMAM G 5.0 (D) with temperature at pH of 5 and 8.



Figure 5. The variation of scattered light intensity of PAMAM-PNIPAM₆₀-PAMAM G 1.0, G 2.0, G 3.0, G 4.0 (A) and PAMAM-PNIPAM₉₀-PAMAM G 1.0, G 2.0, G 3.0, G 4.0 (D) with temperature at pH of 5.

inside the dendrons are nonprotonated, and PAMAM dendrons aggregated into small particles at 25 °C; subsequently, PNIPAM also became hydrophobic and aggregated, forming bigger particles. The scattering intensity increases abruptly between 40 and 41 °C; this observation is in agreement with published evidence.³¹ The results showed that the DPs of PNIPAM had little influence about the LCST. Therefore, the thermosensitivities of the two polymers are similar. Based on the results, we also investigated the temperature-responsive behaviors of PAMAM-PNIPAM-PAMAM G 1.0, G 2.0, G 3.0, and G 4.0 at pH 5 (Figure 5). The LCSTs of these polymers were similar to PAMAM-PNIPAM-PAMAM G 5.0, indicating that the generation of dendrons had little influence on their thermosensitivities.

In this study, we have shown a facile method to synthesize well-defined dendritic–linear–dendritic polymers via RAFT polymerization, aminolysis of the trithiocarbonate, thiol-Michael addition reaction, and amidation, followed by continuous Michael addition and amidation reaction. Also, the temperatureand pH-responsive behaviors of these polymers were investigated. With this method, we are currently synthesizing more dendritic–linear–dendritic copolymers and investigating their applications in drug and gene delivery. The authors gratefully acknowledge support from the National Science Foundation of China (Nos. 51273187, 21374107, and 21474097) and the Fundamental Research Funds of the Central Universities (No. WK2060200012).

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