Synthesis of Temperature-Responsive Heterobifunctional Block Copolymers of Poly(ethylene glycol) and Poly(*N*-isopropylacrylamide)

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Heterobifunctional block copolymers of poly(ethylene glycol) (PEG) and poly(*N*-isopropylacrylamide) (PNIPAM) were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization of NIPAM using a macromolecular trithiocarbonate PEG-based chain transfer agent. The polymerization showed all the expected features of living radical polymerization and allowed the synthesis of copolymers with different lengths of the PNIPAM block. The synthesized block copolymers contained a carboxylic acid group from L-lysine at the focal point and a trithiocarbonate group at the terminus of the PNIPAM block. The trithiocarbonate functionality was converted into a thiol group and used for conjugation of biotin to the end of the PNIPAM block. The copolymers exhibited temperature-dependent association behavior in aqueous solution with a phase transition of approximately 32 °C. The described heterobifunctional block copolymers show promise for surface modifications with the potential for stimulus-controlled surface presentation of ligands attached to the terminus of the PNIPAM block.

Introduction

The ability to reversibly switch and control surface properties via the use of stimuli-responsive polymers offers a great promise in designing drug and gene delivery systems and a range of stimuli-responsive devices and sensors.[1,2] We are particularly interested in stimulus-controlled presentation of targeting ligands in drug and gene delivery vectors. The evidence suggests that so-called Y-shaped AB block copolymers can offer outstanding switching ability.[3] The Y-shaped copolymers consist of two different polymer blocks attached to a single focal point capable of chemical grafting to surfaces.[4] Such copolymers have many potential applications in fabrication of functional surfaces of particles and a variety of other substrates.[3−7] According to theoretical predictions, such Y-shaped molecules can form a wide variety of segregated layers with various micellar surface structures controlled by chemical attachment, grafting density, and composition of the A and B polymer chains. However, only a limited number of such Y-shaped AB copolymers have been described so far.[3,5,8,9]

Water-soluble polymers that undergo phase transitions in response to stimuli such as temperature and pH are widely investigated in drug delivery.[10] These polymers represent ideal candidates for the synthesis of the Y-shaped block copolymers. Poly(*N*-isopropylacrylamide) (PNIPAM) is probably the best-known temperature-sensitive polymer that exhibits a lower critical solution temperature (LCST) in water at 32 °C.[11,12] The LCST of PNIPAM can be easily tuned to a desired temperature by copolymerization with suitable comonomers. Copolymerization with a pH-sensitive comonomer yields NIPAM copolymers in which the phase transition can be triggered by a change in the pH at specific temperature.[13−15] The traditional way of preparing NIPAM copolymers by free radical polymerization leads to polymers with high polydispersity and with poorly defined end-group chemistries, both of which severely limit the possibility of synthesizing well-defined Y-shaped block copolymers. The emergence during the past several years of reversible addition-fragmentation chain transfer (RAFT) polymerization[16] as an exceptionally versatile tool for the synthesis of α,ω-functionalized polymers of narrow molecular weight distributions has opened new possibilities for the synthesis of previously unattainable NIPAM copolymers.[17,18]

Here, we report a synthesis of Y-shaped AB copolymers consisting of one block of poly(ethylene glycol) (PEG) and one block of end-functionalized PNIPAM with lysine as the focal point. The copolymers are synthesized using a macromolecular PEG-based RAFT agent. The terminus of the PNIPAM block is functionalized with biotin. Such heterobifunctional block copolymers show promise for surface modifications with the potential of stimulus-controlled surface presentation of ligands attached to the terminus of the PNIPAM block.

Experimental Section

Materials. Poly(ethylene glycol) methyl ether (mPEG, $M_n = 2000$, Aldrich), disuccinimidyl carbonate (DSC, Fluka, ≥95.0%), 4-(dimethylamino)pyridine (DMAP, 99%), *N*-hydroxysuccinimide (Fluka, ≥97.0%), *N*,*N*′-dicyclohexylcarbodiimide (DCC, Fluka, ≥99.0%), L-lysine (Sigma, 98%), 2,2′-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%), hexylamine (Aldrich, 99%), triethylamine (TEA, Aldrich, 99.5%), 1-dodecanethiol (Aldrich ≥98.5%), tricaprylylmethylammonium chloride (Aliquat 336, Aldrich), carbon disulfide (Sigma-Aldrich, ≥99.9%), 4′-hydroxyazobenzene-2-carboxylic acid (HABA), avidin (Sigma-Aldrich), 1-biotinamido-4-[4′-(maleimidomethyl) cyclohexanecarboxamido]butane (Biotin-BMCC, Pierce), and D-biotin (Sigma) were used directly. *N*-Isopropylacryamide (NIPAM) was recrystallized from hexane. All other reagents and solvents were purchased from Sigma-Aldrich and used without further purification.

Instrumentation. NMR spectra were recorded on a Varian spectrometer (400 MHz). The number-average (M_n) and weight-average (M_w) molecular weight and polydispersity index (PDI, M_w/M_n) of the polymers were determined by size exclusion chromatography (SEC) using a Shimadzu LC-10ADVP liquid chromatograph equipped with a CTO-10ASVP Shimadzu column oven and a Polymer Labs PL gel 5

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μm mixed C column. The system was equipped with a seven-angle BIMwA static light scattering detector and BIDNDC differential refractometer (both from Brookhaven Instruments, Inc.). The BIMwA detector was equipped with a 30 mW vertically polarized solid-state laser (660 nm) as a light source. *N*,*N*-Dimethylformamide (DMF) was used as an eluent at a flow rate of 1.0 mL/min and a temperature of 35 °C. SEC data were analyzed using PSS WinGPC Unity software from Polymer Standards Services. Refractive index increment (dn/dc) of PEO-*b*-PNIPAM was determined with a BIDNDC differential refractometer (Brookhaven Instruments) and used in SEC analysis. The determination of hydrodynamic diameters was performed by dynamic light scattering using a ZetaPlus particle size analyzer.

Synthesis of 2-Dodecylsulfanylthiocarbonylsulfanyl-2-methyl Propionic Acid (DMP).[19] 1-Dodecanethiol (20.2 g, 0.10 mol), acetone (58.0 g, 1.0 mol), and tricaprylylmethylammonium chloride (1.0 g, 0.0025 mol) were added into a flask and cooled to 0 °C under a nitrogen atmosphere. Sodium hydroxide solution (50%) (4.5 g, 0.11 mol) was added over 10 min. After the mixture was stirred for an additional 20 min, carbon disulfide (7.6 g, 0.10 mol) in acetone (10.0 g) was added over 30 min, and the color turned gradually red. Chloroform (17.8 g, 0.15 mol) was added, followed by dropwise addition of 40 g of 50% sodium hydroxide solution over 20 min. The mixture was stirred overnight. Water (200 mL) was added, followed by 80 mL of concentrated HCl to acidify the aqueous solution. After removal of the organic solvents, the solid was filtered and then stirred in 300 mL of isopropanol. The insoluble solid was filtered off. The remaining isopropanol solution was concentrated, and the resulting solid was recrystallized from hexane to afford 12.0 g of the product (yield 33%). ^{1}H NMR ($CDCl_3$, δ): 0.89 (t, 3H, $-C_{11}H_{22}CH_3$), 1.37−1.47 (m, 18H, $-CH_2CH_2C_9H_{18}CH_3$), 1.68 (m, 2H, $-S-CH_2CH_2C_{10}H_{21}$), 1.73 (s, 6H, $-S-C(CH_3)_2COOH$), 3.2 (t, 2H, $-S-CH_2C_{11}H_{23}$).

Synthesis of *N*-Hydroxysuccinimidyl Ester of DMP (DMP-OSu).[20] DMP (2.1 g, 5.7 mmol) and *N*-hydroxysuccinimide (1.0 g, 10.0 mmol) were dissolved in 50 mL of anhydrous dichloride methylene. Dicyclohexylcarbodiimide (DCC, 2.1 g, 10.0 mmol) was added in one portion to the solution. The reaction mixture was stirred at room temperature in the dark for 24 h. A white byproduct was removed by filtration. The filtrate was concentrated by evaporation, and the residue was purified by a silica gel chromatography with ethyl acetate/hexane (1:4, v/v) as the eluent. A yellow solid was obtained (2.0 g, yield 74%). ^{1}H NMR ($CDCl_3$, δ): 0.9 (t, 3H, $-C_{11}H_{22}CH_3$), 1.3−1.5 (m, 18H, $-CH_2CH_2C_9H_{18}CH_3$), 1.68 (m, 2H, $-S-CH_2CH_2C_{10}H_{21}$), 1.75 (s, 6H, $-S-C(CH_3)_2COOH$), 2.80 (s, 4H, $-CH_2CH_2-$), 3.20 (t, 2H, $-S-CH_2C_{11}H_{23}$).

Synthesis of α-Succinimidyl Carbonate-ω-methoxy PEG (mPEG-OSu).[21,22] mPEG-OH (M_n = 2000, PDI = 1.04, 10 g, 5.0 mmol) was dried by azeotropic distillation of toluene and then dissolved in 200 mL of anhydrous CH_3CN, and 7.68 g (30.0 mmol) of DSC and 8.3 mL (60.0 mmol) of triethylamine (TEA) were added with stirring. After 12 h at room temperature, the solution was concentrated under vacuum to approximately 50 mL, and the polymer was isolated by precipitation into 500 mL of diethyl ether. After filtration, the precipitate was dissolved in 60 mL of toluene at 40 °C, and the insoluble fraction was removed by rapid filtration. The solution was concentrated to approximately one-third of its original volume and precipitated in diethyl ether. mPEG-OSu was further purified by dissolution/precipitation with dichloromethane/ether twice (overall yield 82%). ^{1}H NMR ($CDCl_3$, δ): 3.35 (s, 3H, CH_3-O-PEG), 3.50−3.75 (bs, $-CH_2CH_2O-$ of PEG main chain), 2.8 (m, 4H, $-OCCH_2CH_2CO-$), 4.45 (t, 2H, PEG$-CH_2CH_2OCO-$).

Synthesis of L-Lysyl-Terminated mPEG (mPEG-Lys). For the coupling of mPEG-OSu with the amino group of lysine, lysine (5.0 g, 34.2 mmol) was dissolved in 200 mL of water at pH 8.0−8.5, and to this solution mPEG-OSu (6.0 g, 2.8 mmol) was added in several aliquots. The pH of the system was maintained at 8.3. After being stirred overnight at room temperature, the reaction mixture was cooled to 0 °C, and the pH of the solution was adjusted to 3.0 with 1.0 M HCl. The solution was extracted four times with chloroform; organic phases were combined and dried with $MgSO_4$. After filtration, the filtrate was concentrated to approximately one-third of its original volume and added dropwise to diethyl ether. The precipitated product was dried to constant weight to give the mPEG-Lys with a 68% yield. ^{1}H NMR (D_2O, δ): 3.24 (s, 3H, CH_3-O- of mPEG), 3.40−3.70 ($-CH_2CH_2O-$, mPEG main chain), 1.2−1.8 (m, 6H, $CH_2CH_2CH_2$ of Lys), 3.0 (m, 2H, CONH$-CH_2$ of Lys), 4.1 (m, 2H, CH_2CH_2OCONH of mPEG).

Synthesis of mPEG-Lys-DMP. For coupling of DMP-OSu with mPEG-Lys, dried mPEG-Lys (0.62 g, 0.27 mmol) was dissolved in 12 mL of anhydrous chloroform containing DMP-OSu (0.48 g, 1.0 mmol) TEA. After 48 h at room temperature, the reaction mixture was precipitated in diethyl ether three times. ^{1}H NMR ($CDCl_3$, δ): 3.4 (s, 3H, CH_3-O- of mPEG), 3.45−3.70 ($-CH_2CH_2O-$, mPEG main chain), 1.2−1.8 (6H, $CH_2CH_2CH_2$ of Lys; 20H, $-S-CH_2C_{10}H_{20}CH_3$), 3.1 (m, 2H, CONH$-CH_2$ of Lys; 2H $-S-CH_2C_{11}H_{23}$), 4.2 (m, 2H, CH_2CH_2OCONH of mPEG; 1H of $-CH-$ of Lys).

RAFT Polymerization of NIPAM Using mPEG-Lys-DMP as the Chain Transfer Agent. NIPAM (500.0 mg), mPEG-Lys-DMP (100.0 mg), AIBN (1.0 mg), and DMF (3.0 mL) were added into an ampule. The reaction mixture was deoxygenated, and the ampule was sealed under vacuum and placed in a thermostated oil bath at 60 °C. The polymerization was stopped at different time points by opening the ampule to air, and the AB block copolymer (mPEG-Lys-*block*-PNIPAM$-S-C(S)-S-C_{12}H_{25}$) was obtained by precipitation into diethyl ether and drying under vacuum for 24 h at room temperature.

Preparation of mPEG-Lys-*block*-PNIPAM-SH. mPEG-Lys-*block*-PNIPAM$-S-C(S)-S-C_{12}H_{25}$ (0.15 g, M_n = 14 200, PDI = 1.03) was dissolved in 3.0 mL of DMF. The solution was deoxygenated by bubbling with a stream of nitrogen for 30 min followed by addition of 5 drops of aqueous $Na_2S_2O_4$ solution (0.5 M) and hexylamine (0.1 g, 1.0 mmol). The reaction was allowed to proceed for 12 h at room temperature. Rapid discoloration of the yellow solution was observed. The final polymer was isolated by precipitation in diethyl ether.

Preparation of mPEG-Lys-*block*-PNIPAM-Biotin. ′Biotin-BMCC was dissolved in phosphate-buffered saline at a concentration of 2.4 mg/mL. Approximately 4 mg of mPEG-Lys-*block*-PNIPAM-SH copolymer was dissolved in 875 μL of 50 mM phosphate buffer (pH 7.0) just prior to the reaction. Biotin-BMCC solution (120 μL) was added to the block copolymer solution, and the reaction was left to proceed overnight at room temperature. The excess Biotin-BMCC was removed by SEC on a PD-10 column (GE Healthcare).

Avidin−HABA Assay. A total of 1.5 mg of avidin was dissolved in 3 mL of 0.1 M sodium phosphate and 0.15 M NaCl (pH 7.4). HABA was dissolved in 10 mM NaOH at a concentration of 2.42 mg/mL (10 mM), and 75 μL of this solution was added to the avidin solution. D-Biotin was dissolved in 0.1 M sodium phosphate and 0.15 M NaCl (pH 7.4) at a concentration of 0.5 mM. This solution was added in 10 μL aliquots to the avidin−HABA complex, while the absorbance at 500 nm was measured after each addition. When the absorbance at 500 nm was plotted versus the amount of biotin added, the calibration curve was constructed. In parallel, incremental amounts of mPEG-Lys-*block*-PNIPAM-Biotin were added to the avidin−HABA solution, and the absorbance at 500 nm was measured. Biotin content was then determined from the calibration curve.

Results and Discussion

Synthesis and Characterization of mPEG-Lys-DMP RAFT Agent. To synthesize the desired heterobifunctional Y-shaped block copolymers with functional groups at a terminus and in between the blocks, the hydroxyl group of mPEG was first activated with disuccinimidyl carbonate. The presence of the succinimidyl carbonate group in purified mPEG-OSu was confirmed by ^{1}H NMR. In the subsequent step, mPEG-OSu was reacted with lysine to introduce amino and carboxylic acid functionalities. Because of the higher reactivity of the ϵ-amino

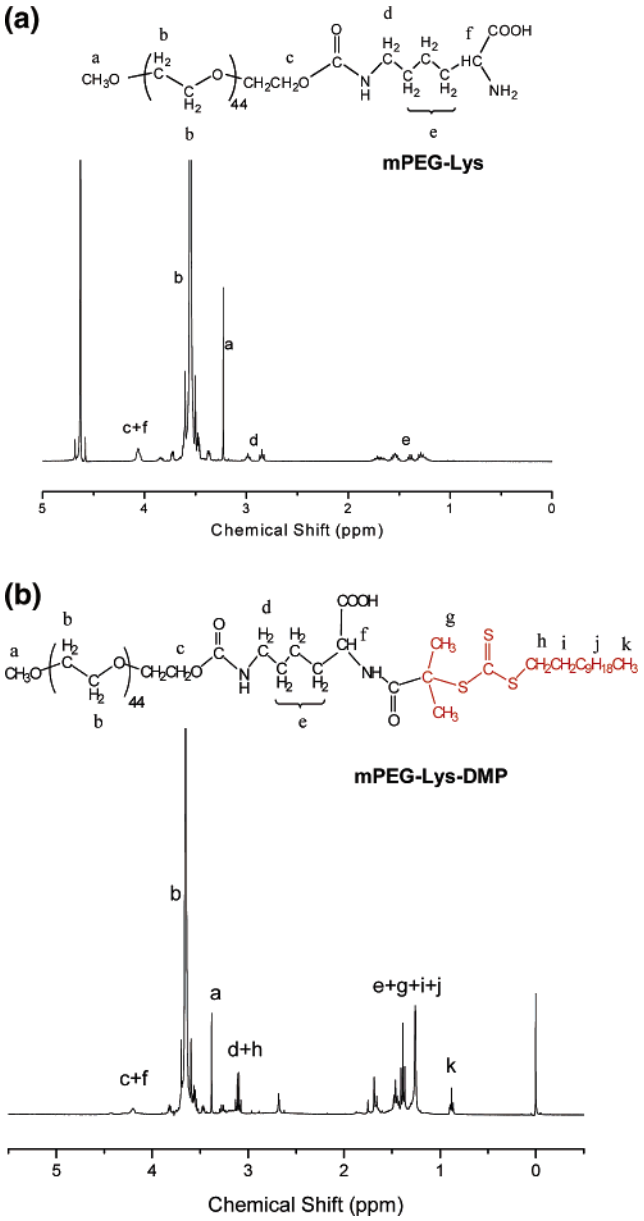


Figure 1. ^{1}H NMR spectra of (a) mPEG-Lys and (b) mPEG-Lys-DMP.

group of Lys as compared to that of its α-amino group, it is expected that when a large excess of Lys is used in the reaction with mPEG-OSu the product will consist of lysyl residues conjugated at the ϵ-amine.[23,24] The appearance of characteristic signals of Lys (1.2−1.8 and 3.0 ppm) in the ^{1}H NMR spectrum of mPEG-Lys (Figure 1a) confirms the desired product. It is evident from the integral ratio of the terminal methyl protons of mPEG (3.24 ppm) to the six methylene protons of Lys (1.2−1.8 ppm) that one mPEG chain contains approximately one lysine group. Further analysis of mPEG-Lys confirms that the molecular weight and polydispersity index were not significantly affected by lysine attachment (Figure 2a). The SEC trace of mPEG-Lys is symmetrical with no signs of a shoulder indicative of two mPEG molecules reacting with one Lys. In addition, the Fourier transform infrared (FTIR) spectrum of mPEG-Lys shows a broad absorption from 1680 to 1740 cm^{-1} assigned to the C═O stretch of lysine −C(O)−NH− and the C═O stretch of lysine COOH (Figure 2b). Taken together, all these facts verify that succinimidyl carbonate-terminated mPEG reacted with only one amino group of lysine. The carboxylic acid group of DMP RAFT agent was activated as *N*-hydroxysuccinimidyl

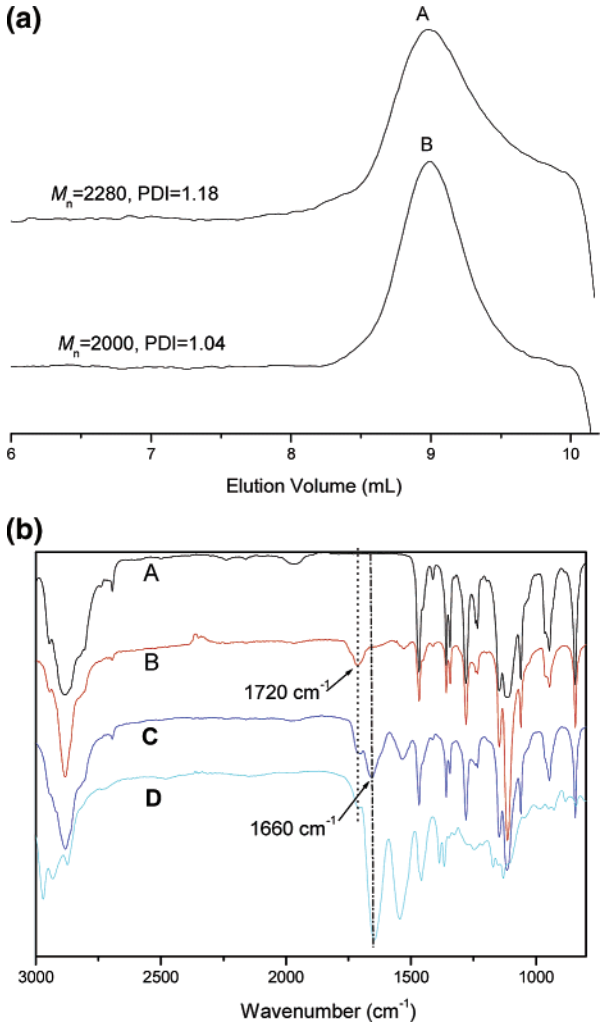
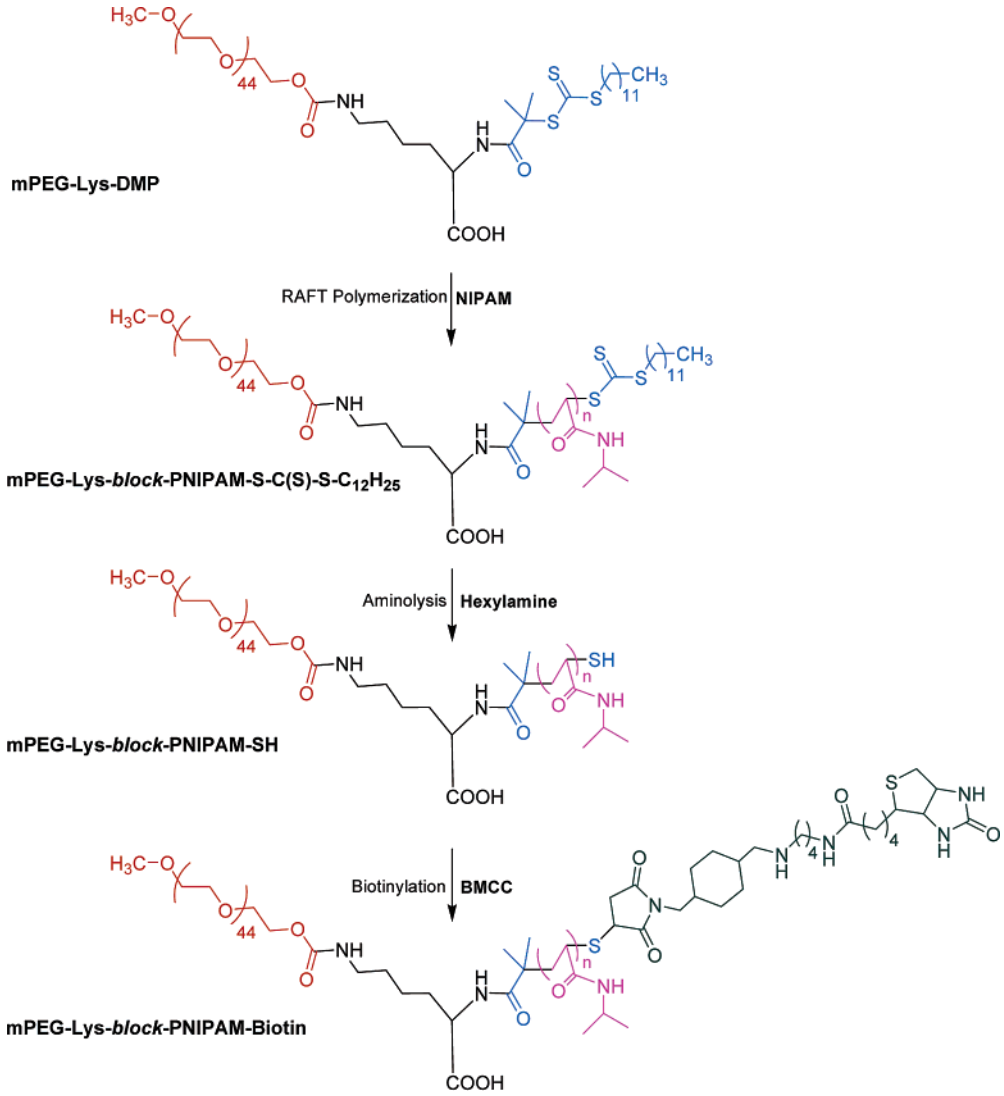


Figure 2. (a) SEC traces of (A) mPEG-OH and (B) mPEG-Lys. (b) FTIR spectra of (A) mPEG-OH, (B) mPEG-Lys, (C) mPEG-Lys-DMP, and (D) mPEG-Lys-*block*-PNIPAM−S−C(S)−S−$C_{12}H_{25}$.

ester and subsequently coupled with the free amino group of mPEG-Lys to produce mPEG-Lys-DMP. The ^{1}H NMR spectrum of the synthesized mPEG-Lys-DMP is shown in Figure 1b. The integral ratio of the terminal methyl protons of mPEG (3.4 ppm) and the methyl protons of DMP (0.90 ppm) was found to be approximately 1.0, which indicates that the DMP functionality has been successfully linked to the N-terminus of mPEG-Lys. In the FTIR spectrum of PEG-Lys-DMP, a new absorption appears at 1660 cm^{-1} corresponding to the C═O stretch absorption of a newly formed amide linkage between DMP and mPEG-Lys (Figure 2b), while the previous lysine absorptions at 1680−1740 cm^{-1} remain unchanged.

RAFT Polymerization of NIPAM Using mPEG-Lys-DMP as the RAFT Agent. RAFT polymerization is one of the most promising living free radical polymerizations, and it is arguably more versatile with respect to monomer choice than other living free radical polymerization techniques as it can be applied to virtually any type of monomer without the necessity of protecting functional groups. RAFT polymerization produces polymers with well-defined reactive end groups and is therefore very effective in preparing block copolymers and other complex polymer structures.[16,17,25−29] Here, we have used mPEG-Lys-DMP as a macromolecular RAFT agent to prepare block copolymers with NIPAM. The polymerization of NIPAM was performed by using AIBN as the initiator, and it resulted in heterobifunctional AB diblock copolymers containing one arm

Scheme 1. Synthetic Pathway for the Heterobifunctional Y-Shaped Block Copolymers mPEG-Lys-*block*-PNIPAM-Biotin



of PEG and one arm of PNIPAM with lysine as the focal point (Scheme 1). The living nature of the polymerization using a mPEG-Lys-DMP RAFT agent was confirmed by the results shown in Figures 3 and 4. Data in Figure 3 show that the M_n values of the produced AB block copolymers increase linearly with increased monomer conversion. The molecular weight distributions of the synthesized block copolymers were narrow (PDI 1.03−1.30) as shown in Figure 3. Block copolymers with varying lengths of the PNIPAM block were also prepared to further demonstrate the living nature of the polymerizations. All of the synthesized copolymers show rather symmetric SEC

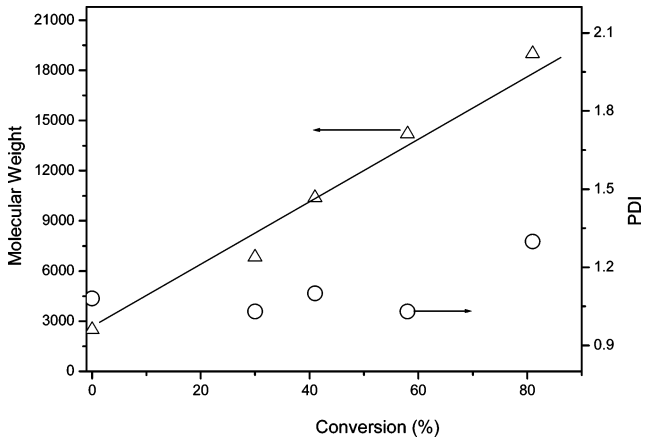


Figure 3. Effect of NIPAM monomer conversion on the molecular weight (M_n) and molecular weight distribution (PDI) of the block copolymers mPEG-Lys-*block*-PNIPAM−S−C(S)−S−$C_{12}H_{25}$.

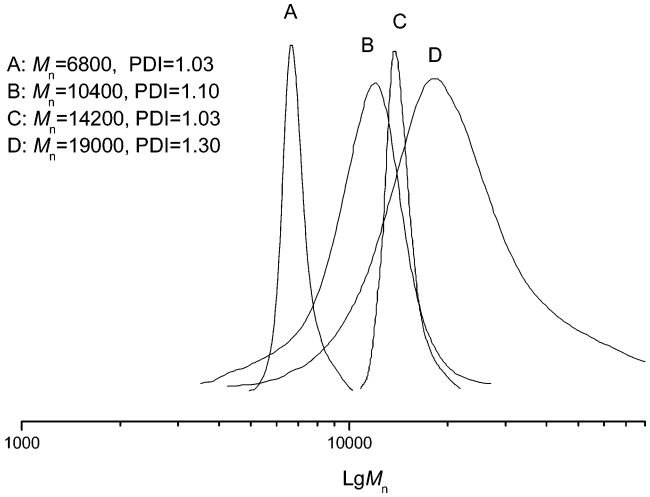


Figure 4. SEC traces of mPEG-Lys-*block*-PNIPAM−S−C(S)−S−$C_{12}H_{25}$ copolymers with different lengths of the PNIPAM block.

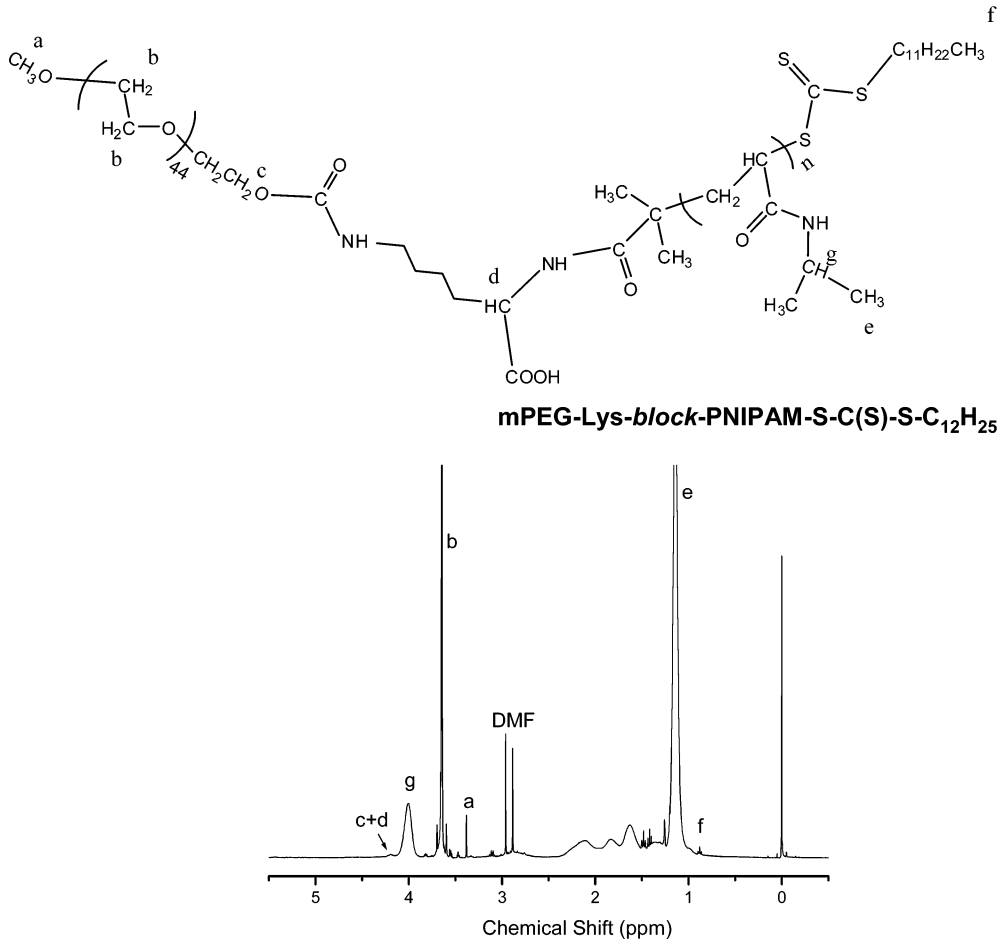


Figure 5. ^{1}H NMR spectrum of mPEG-Lys-*block*-PNIPAM−S−C(S)−S−$C_{12}H_{25}$.

traces (Figure 4). All of these facts indicate that mPEG-Lys-DMP is an effective macro-RAFT agent for the polymerization of NIPAM.

The synthesized copolymers were further characterized by ^{1}H NMR and FTIR spectral data (Figures 5 and 2b). The NMR spectrum of the block copolymers confirms the presence of all of the expected functionalities. These include a broad signal at 3.6 ppm (main chain methylene protons of mPEG), a singlet at 3.4 ppm (terminal methyl protons of mPEG), a broad signal at 4.0 ppm (methane protons of PNIPAM), and a broad signal at 1.2 ppm (methyl protons of PNIPAM). Moreover, a triplet at 0.88 ppm due to the terminal methyl protons of DMP is also clearly visible. The methylene protons of the lysine and the signal of the $C_{11}H_{22}$ group are masked by methine and methylene protons of the PNIPAM backbone.

Synthesis of mPEG-Lys-*block*-PNIPAM-Biotin Copolymers. The trithiocarbonate residue from the RAFT agent at the terminus of the PNIPAM block of the synthesized copolymers can be easily converted into a thiol group, which can be then utilized in a variety of subsequent conjugation reactions. It is well-known that reaction between thioesters and primary amines occurs rapidly at ambient temperatures, leading to the formation of thioamides and thiols.[30−34] In this work, the synthesized mPEG-Lys-*block*-PNIPAM−S−C(S)-S−$C_{12}H_{25}$ block copolymers containing a trithiocarbonate-terminated PNIPAM block were subjected to aminolysis with hexylamine (Scheme 1). The addition of hexylamine to a solution of the copolymers was accompanied by a rapid color change from yellow to pale yellow, indicating the aminolysis of the terminal trithiocarbonate functionality. The trithiocarbonate moiety has a strong absorption band at 320 nm, which allows one to use UV−vis spectroscopy as a simple procedure to follow the extent of the reaction. As a result of the aminolysis, the absorption due to the trithiocarbonate moiety was absent within 90 min of the reaction, indicating a quantitative conversion to the thiol group. The basic conditions of the aminolysis reaction leave the newly formed thiol groups highly susceptible to oxidation and thus to a coupling of two block copolymers. To avoid the oxidative coupling of the thiol terminus, a small amount of antioxidant in the form of aqueous sodium bisulfite ($Na_2S_2O_4$) was added to the aminolysis reaction. Consequently, the formation of disulfides that result from oxidative coupling of thiol end groups could be effectively suppressed as documented in Figure 6.[32,35] The data show that there is no significant change in M_n and PDI values before (PDI = 1.03, M_n = 14 200) and after (PDI = 1.28, M_n = 13 090) aminolysis, confirming an effective suppression of the oxidative coupling reaction.

The terminal thiol group of the synthesized heterobifunctional mPEG-Lys-*block*-PNIPAM-SH copolymers can be utilized for conjugation of a wide range of ligands. Here, we have utilized the terminal thiol group for the coupling of biotin to synthesize mPEG-Lys-*block*-PNIPAM-Biotin copolymers. We used the maleimide-containing biotin reagent BMCC for the coupling due to the easy and irreversible nature of the reaction. The efficiency of the coupling reaction was determined by a colorimetric HABA−avidin assay and by ^{1}H NMR spectroscopy. The average biotinylation efficiency determined from the HABA assay was 52%, while the efficiency calculated from NMR was 79%. Similar efficiencies have been previously reported.[36]

Properties of mPEG-Lys-*block*-PNIPAM-Biotin Copolymers. PNIPAM is a "smart" polymer whose conformation and

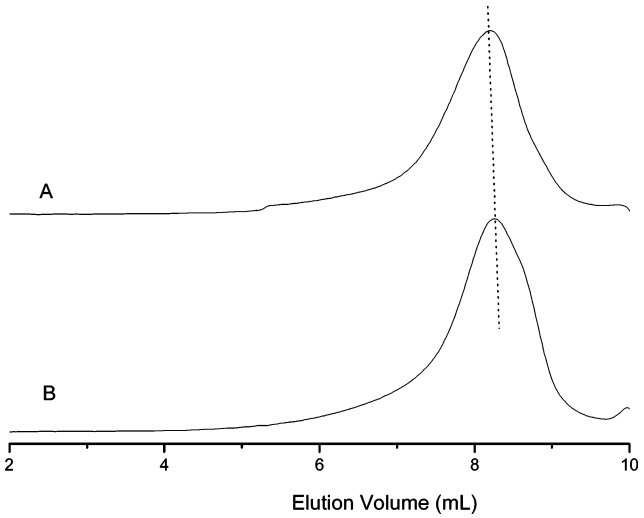


Figure 6. SEC traces of (A) mPEG-Lys-*block*-PNIPAM−S−C(S)-S−$C_{12}H_{25}$ and (B) mPEG-Lys-*block*-PNIPAM-SH.

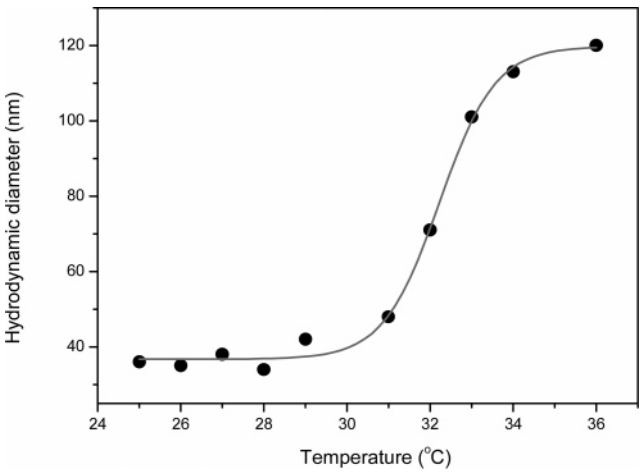
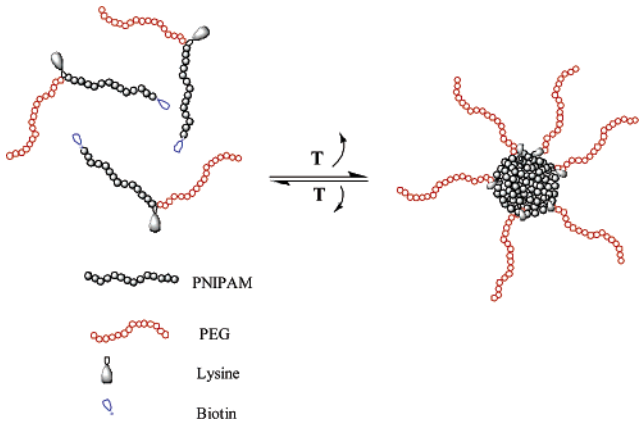


Figure 7. Temperature-induced association of mPEG-Lys-*block*-PNIPAM-Biotin copolymer (M_n = 13 000).

solubility in water changes abruptly with temperature. At temperatures below its lower critical solution temperature (LCST), the intermolecular hydrogen bonding between the PNIPAM chains and water molecules is dominant, and the PNIPAM chains are soluble in water. At temperatures above the LCST, intramolecular hydrogen bonding between C═O and N−H groups in PNIPAM results in a transition into compact and collapsed conformations of PNIPAM chains, and PNIPAM is insoluble in water. To confirm the LCST behavior of the synthesized mPEG-Lys-*block*-PNIPAM-Biotin copolymers, we have measured the temperature dependence of the hydrodynamic diameter in an aqueous solution of the copolymers. Dynamic light scattering was used to determine the changes in the diameter of the copolymers with the temperature increasing from 25 to 35 °C (Figure 7). The results show that the hydrodynamic diameter of the copolymers does not change much below 31 °C but a sharp increase is observed above 31 °C, corresponding to the phase transition of PNIPAM from soluble to insoluble form. The PNIPAM chains collapse to form a nanoparticle core while the PEG chains remain soluble and form a shell to stabilize the nanoparticles/micelles as shown in Scheme 2. This phenomenon is similar to previous research on PEG-*b*-PNIPAM.[37−41]

On the basis of the anticipated core−shell structure of the copolymer particles formed above the LCST, it was expected that the biotin groups located in the cores of the particles will exhibit a limited accessibility for interactions with avidin. This

Scheme 2. Schematic Representation of Temperature-Induced Association of the mPEG-Lys-*block*-PNIPAM-Biotin Copolymers



prediction was confirmed by measuring the interaction of the copolymers with HABA−avidin complexes. At temperatures above the LCST, the measured biotin content was only approximately 10% of the value determined below the LCST. This observation confirms the limited accessibility of biotin above the LCST and the possibility to control the presentation of terminal ligands in the mPEG-Lys-*block*-PNIPAM copolymers. The interaction of biotin in the block copolymers with avidin was further studied by dynamic light scattering (Figure 8). Avidin contains four biotin binding sites, and thus up to four block copolymer molecules can bind to avidin. The hydrodynamic diameter was measured in a solution of mPEG-Lys-*block*-PNIPAM-Biotin before and after addition of avidin (final molar ratio copolymer/avidin = 4:1). Before addition of avidin, the diameter of the copolymer was approximately 31 nm. The diameter increased to 135 nm within 10 min of the addition of avidin, clearly indicating binding of multiple block copolymer molecules to avidin.

Conclusions

The RAFT functionality was successfully linked to a terminus of mPEG functionalized with lysine. Heterobifunctional AB block copolymers containing PEG and PNIPAM blocks and a lysine focal point were synthesized by RAFT polymerization of NIPAM using a mPEG-based macro-RAFT agent. Block copolymers with thiol termini were produced by treating the

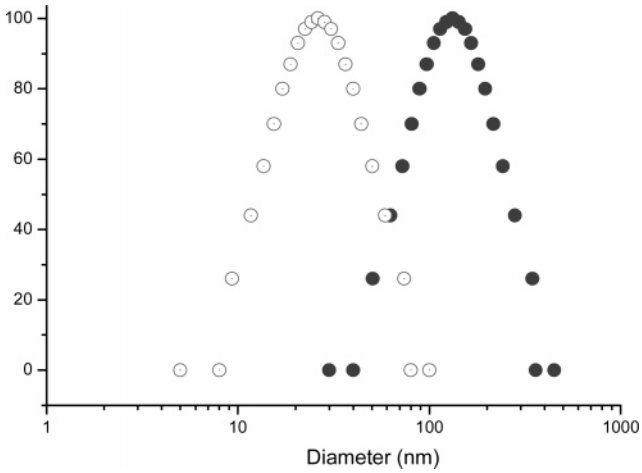


Figure 8. Log-normal size distribution of mPEG-Lys-*block*-PNIPAM-Biotin copolymer (○) and mPEG-Lys-*block*-PNIPAM-Biotin copolymer complex with avidin (●) determined at 25 °C.

copolymers with hexylamine. The terminal thiol group was then utilized for attachment of biotin, resulting in block copolymers containing PEG and PNIPAM blocks with a reactive carboxylic acid group located between the two blocks and with a biotin group at the end of the PNIPAM block. As expected, these copolymers showed temperature sensitivity and formed complexes with avidin. The carboxylic acid group located between the two blocks should allow an easy attachment of these copolymers to the surfaces of particles and a variety of substrates. The presence of the PEG and PNIPAM blocks should then allow selective presentation of ligands attached to the termini of the PNIPAM blocks by temperature or other stimuli. Overall, the heterobifunctional block copolymers synthesized in this study are expected to find use in a variety of biomedical applications in which stimulus-controlled presentation of ligands is desired.

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