Thermosensitive Block Copolymers Consisting of Poly(*N*-isopropylacrylamide) and Star Shape Oligo(ethylene oxide)

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Thermosensitive block copolymers of ethylene oxide and *N*-isopropylacrylamide (NIPAM) were synthesized. A five armed star shape oligo(ethylene oxide) initiator with a cyclotriphosphazene core was prepared and used for the atom transfer radical polymerization (ATRP) of NIPAM. The lower critical solution temperatures (LCSTs) of the copolymers were 36 to 46 °C, higher than that of PNIPAM (32 °C), depending on their molecular weights. The copolymers were soluble in water below the LCSTs but formed micelles above the LCSTs. The thermosensitive micellization behaviors of the polymers were investigated by fluorescence spectroscopy. With increasing the temperature of an aqueous solution of **P2** and pyrene above the LCST, the peak of 333 nm red-shifted to appear around 339 nm and its intensity increased significantly, indicating the micelle formation. The transfer of pyrene into the micelles was also confirmed by a confocal laser scanning microscope. The fluorescence image obtained from **P2** in an aqueous pyrene solution exhibited a green emission resulting from the pyrene transferred into the micelles. Salt effects on the solubility of the copolymers in an aqueous solution were investigated. The LCST of **P2** decreased sharply as the concentration of sodium chloride increased, while decreased slowly with potassium chloride.

Key Words: Block copolymers, Thermosensitive polymers, Lower critical solution temperature, Cyclotriphosphazene

Introduction

Water soluble thermosensitive polymers that show a lower critical solution temperature (LCST) have widely been investigated due to their potential applications such as controlled drug delivery,[1] biomimetic actuators,[2] chromatographic separations,[3] gene-transfection agents,[4] and immobilized biocatalysts.[5] Below the LCST the polymer molecules exist in an aqueous solution as extended coils, surrounded by ordered water molecules, while above the LCST the polymer precipitates out of the solution. Block copolymers of water soluble thermo-responsive polymers having different hydrophilic blocks have also attracted considerable attention because of their unique self aggregation behaviors depending on a temperature as well as a concentration. These double hydrophilic block copolymers dissolve in water homogeneously below the LCSTs of the thermo-responsive blocks and behave like amphiphilic block copolymers above the LCSTs.[6]

Poly(ethylene oxide)-poly(*N*-isopropylacrylamide) (PEO-PNIPAM) is one of the typical double hydrophilic block copolymers. PNIPAM is a well known thermosensitive polymer, which exhibits a rapid and reversible hydration-dehydration change in response to small temperature cycles around its LCST (32 °C). PEO shows excellent biocompatibility and water solubility. The thermally induced micellization of PEG-*b*-PNIPAM has been studied by several groups.[7-14] Below the LCST of PNIPAM, both blocks are hydrophilic and soluble in water. Above the LCST of PNIPAM, the copolymer becomes amphiphilic to form a micelle.

Another interesting property of PEO is its ability to capture metal cations. The complexation of PEO considers weaker and less selective than macrocyclic ethers such as crown ethers.[15] but some chemically modified PEOs with structures mimicking natural acyclic polyethers showed fairly good complexation ability and selectivity.[16,17] Oligo(ethylene oxide) or crown ether bearing polyphosphazenes were also used for cation complexation.[18-20]

In this paper, we report thermoresponsive polymers consisting of oligo(ethylene oxide) and poly(*N*-isopropyl acrylamide) blocks. Cyclotriphosphazenes are versatile starting materials for the synthesis of multifunctional cyclic compounds.[21,22] We prepared a cyclotriphosphazene initiator having five oligo (ethylene oxide) substituents and used for the atom transfer radical polymerization (ATRP) of NIPAM. The resulting copolymers had a star shape oligo(ethylene oxide) block with a polypodand structure, which were expected to capture metal cations.

Experimental Section

Materials and Instruments. Hexachlorocyclotriphosphazene, 2-bromoisobutyryl bromide, tris(2-aminoethyl)amine, and 4-hydroxybenzaldehyde were purchased from Aldrich Chemical Co. and used as received. Tetrahydrofuran (THF) was dried over sodium metal and distilled. Di(ethylene oxide) monomethyl ether was purified by azeotropic distillation with benzene. *N*-Isopropylacrylamide (NIPAM) was purified by recrystallization from *n*-hexane and dried carefully in a vacuum. Tris(2-dimethylaminoethyl)amine (Me_6TREN) was synthesized by following the method of Ciampolini.[23] Nuclear magnetic resonance (NMR) spectra were measured by a Bruker Avance DPX-300 (300 MHz for ^{1}H NMR) spectrometer and a

Bruker Avance DPX-500 (200 MHz for ^{31}P NMR) spectrometer. Elemental analyses were carried out on a Flash EA 1112 elemental analyzer. Fluorescence spectra were recorded on a RF-5301 spectrafluorophotometer (Shimadzu) with a 150 W xenon lamp and a 1 cm quartz cell. Gel permeation chromatographic (GPC) analysis was conducted in THF at 35 °C with a M930 solvent delivery system and a RI750F refractive index detector from Younglin, equipped with Styragel HR 5E and Styragel HR 6 columns from Waters. Approximate calibration of the column was accomplished by means of narrow molecular weight polystyrene standards. The cloud points of the copolymers were determined by turbidity measurement using a Sinco S-3150 UV-visible spectrophotometer. The cloud point was defined as the temperature at the inflection point in the absorbance of a polymer solution (0.2 wt %), which was measured at 450 nm while the temperature was raised from 20 to 60 °C at increment of 0.2 °C/min. Confocal laser scanning microscopy study was performed by a radiance 2000/MP (Bio-RAD), equipped with an IR laser as the light source.

Synthesis of Compound 2. A solution of diethylene oxide monomethylether (3.30 g, 27.5 mmol) in 100 mL of THF was added dropwise to a suspension of sodium hydride (0.72 g, 30.0 mmol) in THF (50 mL) at room temperature. To the solution was added a solution of hexachlorocyclotriphosphazene (1.74 g, 5.0 mmol) in THF (100 mL) at room temperature and the mixture was stirred at reflux temperature for 48 h. After filtration using celite and evaporation, the crude product was isolated by column chromatography on silica gel (chloroform/methanol = 19/1 v/v) and used for the next reaction without further purification.

A solution of 4-hydroxybenzaldehyde (0.61 g, 5.0 mmol) in 50 mL of THF was added dropwise to a suspension of sodium hydride (0.15 g, 6.0 mmol) in THF (50 mL) at room temperature. To the solution was added a solution of compound **1** (1.74 g, 5.0 mmol) in THF (100 mL) at room temperature and the mixture was stirred at reflux temperature for 24 h. After filtration using celite, the solvent was evaporated and the compound **2** was isolated by column chromatography on silica gel (chloroform/methanol = 24/1 v/v). Yield: 1.96 g (46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.97(s, -CHO), 7.40-7.95 (dd, Ar-H), 3.22-4.58 (m, CH_2). ^{31}P NMR (200 MHz, DMSO-d_6) δ 17.8 (d, DEO-P-DEO), 14.5 (t, DEO- P-OAr). Anal. Calcd (in wt%) for $C_{32}H_{60}N_3O_{17}P_3$: C, 45.12; H, 7.10; N, 4.93. Found: C, 45.14; H, 7.21; N, 4.85.

Synthesis of Compound 3. To a solution of compound **2** (2.00 g, 2.4 mmol) in THF-methanol (100 mL, 1:1, v/v) was added sodium borohydride (0.10 g, 2.5 mmol) at room temperature. After stirring for 24 h at room temperature, distilled water was added dropwise until the precipitates were dissolved. The reaction mixture was concentrated by evaporation under reduced pressure. Compound **3** was isolated by column chromatography on silica gel (methylene chloride/methanol = 19/1 v/v). Yield: 1.88 g (92%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 6.95, 7.79 (dd, 4H, Ar-H), 5.20 (t, 1H, OH), 4.50 (d, 2H, CH_2), 3.31-4.02 (m, CH_2 in diethylene oxide). ^{31}P-NMR (200 MHz, DMSO-d_6) δ 14.5 (t, DEO-P-OAr), 17.8 (d, DEO-P-DEO). Anal. Calcd (in wt%) for $C_{32}H_{62}N_3O_{17}P_3$: C, 45.02; H, 7.32; N, 4.92. Found: C, 45.05; H, 7.31; N, 4.83.

Synthesis of Star Shape Oligo(ethylene oxide) Initiaor (4). To a solution of compound **3** (1.00 g, 1.2 mmol) in THF (50 mL) were added triethylamine (0.24 g, 2.3 mmol) and 2-bromoisobutylbromide (0.27 g, 1.2 mmol). After stirring for 6 h at room temperature, the solvent was evaporated and the product was isolated by column chromatography on silica gel (chloroform/methanol = 19/1 v/v). Yield: 1.14 g (95%). ^{1}H- NMR (300 MHz, DMSO-d_6) δ 7.30, 7.16 (dd, 4H, ArH), 5.15 (s, 2H, CH_2), 3.31-4.02 (m, 40H, CH_2 in ethyleneglycol protons), 3.18 (s, 15H, CH_3 of diethyleneglycol), 1.90 (s, 6H, $(CH_3)_2$). ^{31}P-NMR (200 MHz, DMSO-d_6) δ 14.5 (t, DEO-P- OAr), 17.8 (d, DEO-P-DEO). Anal. Calcd (in wt%) for $C_{36}H_{67}BrN_3O_{18}P_3$: C, 43.12; H, 6.73; N, 4.19. Found: C, 42.95; H, 6.62; N, 4.06.

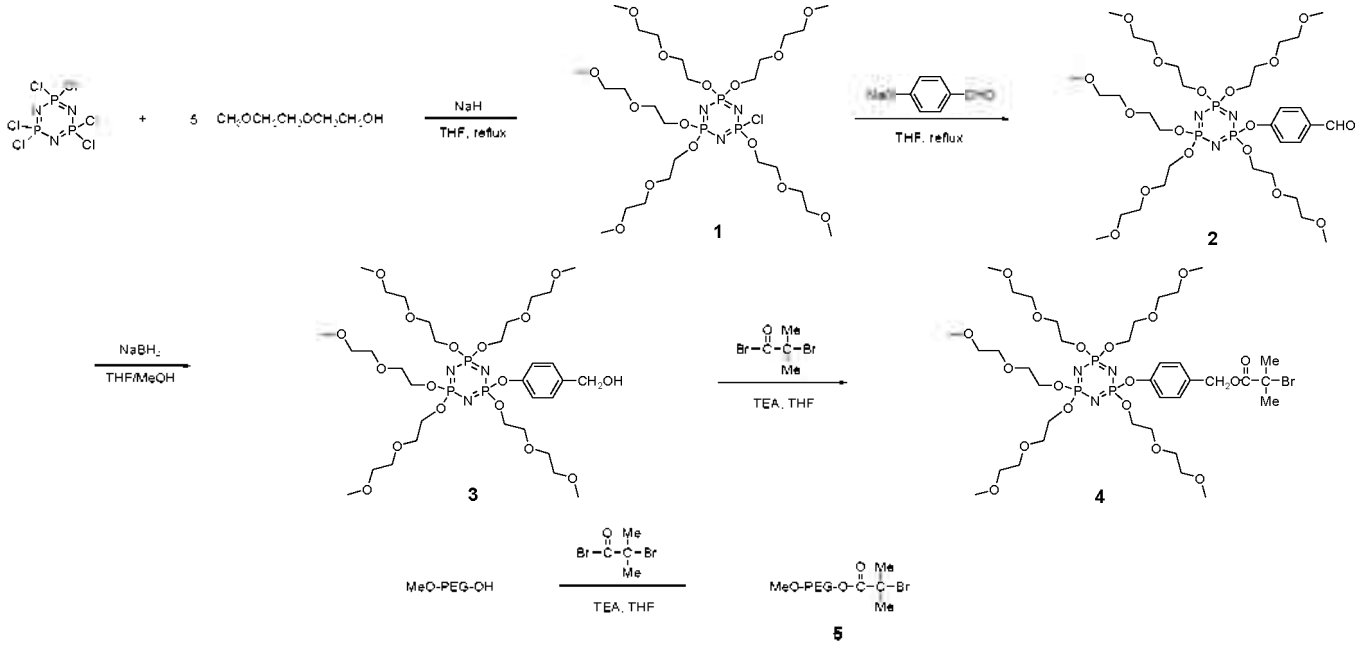
Preparation of Linear PEG Initiator (5). Triethylamine (0.6 g, 6 mmol) was added to a solution of polyethylene oxide metyl ether (M_n = 750) (1.5 g, 2 mmol) in methylene chloride (50 mL). After stirring for 2 h at room temperature, 2-bromoisobutyryl bromide (0.50 g, 2.2 mmol) was added. The reaction mixture was stirred for 6 h at room temperature. After filtration, the filtrate was concentrated by evaporation under reduced pressure. The product was isolated by column chromatography on silica gel (CH_2Cl_2/MeOH = 24/1 v/v) as an oil. Yield: 1.17 g (65%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 4.3 (t, 2H, CH_2), 3.5 (m, CH_2 in ethylene oxide protons), 1.9 (s, 6H, $(CH_3)_2$).

Synthesis of starEO-*b*-PNIPAM and PEG-*b*-PNIPAM. Initiator **4** (0.10 g, 0.1 mmol) and *N*-isopropylacrylamide (0.57 g, 5.0 mmol) were dissolved in 3 mL of water/DMF (2/1, v/v) in an ampule (10 mL). A solution of Me_6TREN (0.023 g, 0.1 mmol) and CuBr (0.014 g, 0.1 mmol) in 1 mL of DMF was added to the initiator/monomer solution. The mixture in the ampule was degassed five times with a freeze-pump-thaw procedure. The ampule was sealed in vacuo and polymerized at 30 °C. After 5 h, the ampule was opened, and the solution was passed through a neutral aluminum oxide column to remove the catalyst. The filtrate was concentrated under reduced pressure and the resulting polymer was isolated by dialysis in water through a cellulose membrane tube with molecular weight cutoff of 2000. The block copolymer was freeze-dried. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.20 (br, NH and aromatic), 4.95 (s, CH_2 in starEO), 3.90 (br, CH in PNIPAM), 3.10-3.60 (m, CH_2 in DEO of starEO), 1.95 (br, CH_2 in PNIPAM), 1.45 (br, CH_2 in PNIPAM), 1.05 (Br, CH_3 in PNIPAM). ^{31}P-NMR (200 MHz, DMSO-d_6) δ 14.5 (t, DEO-P-OAr), 17.8 (d, DEO-P-DEO).

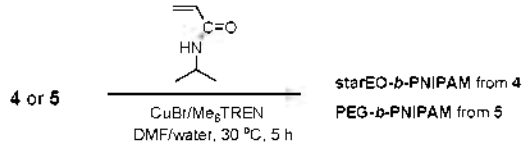
Linear PEG-*b*-PNIPAM was also prepared in the same manner except that PEG initiator **5** was used instead of **4**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.20 (br, NH in PNIPAM), 3.90 (br, CH in PNIPAM), 3.50 (br, CH_2 in PEG), 1.95 (br, CH_2 in PNIPAM), 1.45 (br, CH_2 in PNIPAM), 1.05 (Br, CH_3 in PNIPAM).

Results and Discussion

Synthesis. A five armed star shape oligo(ethylene oxide) (**starEO**) initiator with a cyclotriphosphazene core for the ATRP of NIPAM was synthesized according to Scheme 1.



Scheme 1



Scheme 2

Table 1. Molecular Weights and LCSTs of the Copolymers.

Copolymer	Initiator	M_n[a]	M_w[a]	LCST (°C)
starEO-*b*-NIPAM (P1)	**4**	5100	5500	46
starEO-*b*-NIPAM (P2)	**4**	6100	6700	38
starEO-*b*-NIPAM (P3)	**4**	7300	7900	36
PEG-*b*-PNIPAM[b]	**5**	7100	7800	39

[a]Measured by GPC in THF with polystyrene standards. [b]M_n of a PEG block = 750.

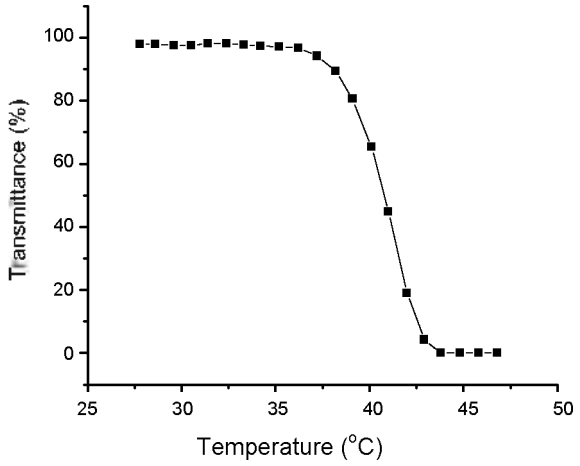


Figure 1. Transmittance *vs* temperature of **P2** in an aqueous solution.

Five chloro groups of hexachlorocyclotriphosphazene were first substituted by sodium salt of methoxyethoxyethoxide. The remaining chloro group was replaced by sodium salt of 4-formylphenoxide. After converting the formyl group into an alcohol with sodium hydride, the resulting compound was reacted with 2-bromoisobutyryl bromide to give macroinitiator **4**. Linear PEO initiator **5** was also prepared by reaction of poly(ethylene oxide) methyl ether (MeO-PEO-OH) (M_n = 750) with 2-bromoisobutyryl bromide.

The structure of compound **4** was confirmed by ^{1}H NMR and ^{31}P NMR spectroscopy. In the ^{1}H NMR spectrum in DMSO-d_6, two doublet peaks for aromatic ring protons appeared at 7.12 and 7.35 ppm and a singlet peak for isobutyrylbromide group protons at 1.95 ppm. The ^{31}P NMR spectrum showed a doublet peak at 17.8 ppm for phosphorous atoms substituted with two methoxyethoxyethoxy groups and a triplet peak at 14.5 ppm for a phosphorous atom substituted with a methoxyethoxyethoxy and a phenoxy group.

N-Isopropylacrylamide was polymerized in DMF/water (1/1 v/v) by ATRP using initiator **4** or **5** and CuBr/ Me_6TREN as a catalyst/ligand system (Scheme 2). The polymerization results are summarized in Table 1.

The LCSTs of the polymers were measured using a UV-visible spectrophotometer. The cloud point was defined as the temperature at the inflection point in the absorbance of a polymer solution (0.2 wt%) at 450 nm on heating (Figure 1). The LCSTs of the copolymers were higher than that of PNIPAM (32 °C) possibly due to the hydrophilic PEG end group. They were also dependent on their molecular weights. When the length of a PNIPMA block increased, the LCST of the polymer decreased (Table 1).[24]

The thermosensitive micellization behaviors of the polymers were investigated. Figure 2(a) shows the fluorescence excitation spectra of pyrene molecules in the polymer solution, which were obtained below and above the LCST. The

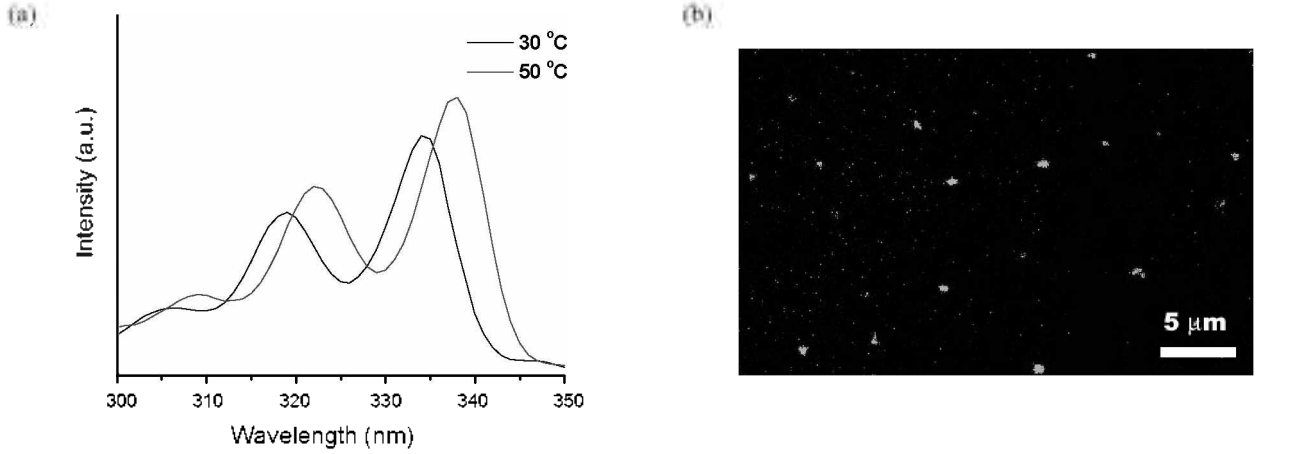


Figure 2. (a) Fluorescence excitation spectra (λ_{em} = 393 nm) of a solution of pyrene and **P2** in water, measured at 30 and 50 °C. (b) CLSM fluorescence image of pyrene-loaded micelles.

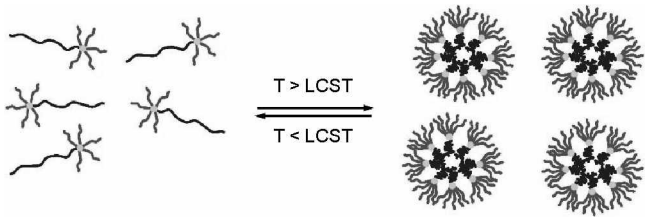


Figure 3. The thermosensitive micellization of **starOE-*b*-PNIPAM**.

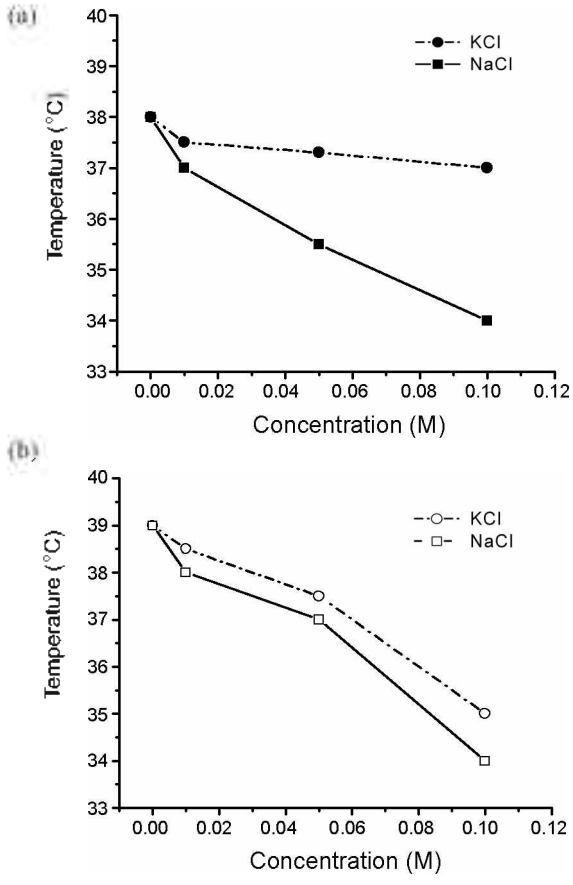


Figure 4. LCST changes of (a) **starEO-*b*-PNIPAM** (**P2**) and (b) linear **PEG-*b*-PNIPAM** according to concentrations of NaCl and KCl.

sample solution was prepared as follows. A solution of pyrene (0.012 mg) in THF (0.12 mL) was added to distilled water (100 mL) and vigorously stirred for 30 min to evaporate THF, resulting in an aqueous pyrene solution (1.2×10^{-6} M). The pyrene solution was mixed with an aqueous solution of **P2** (2 mg/mL) in the same amounts. With increasing the temperature above the LCST, the peak of 333 nm red-shifted to appear around 339 nm and its intensity increased significantly, indicating the micelle formation. Below the LCST of **P2**, pyrene molecules and the copolymer made a homogeneous solution. Above LCST, the PNIPAM block became hydrophobic, while the oligo(ethylene oxide) block remained hydrophilic. As a result, the copolymer formed a micelle so that pyrene molecules transferred into the hydrophobic interior (Figure 3). The transfer of pyrene into the **starEO-*b*-PNIPAM** micelles was also confirmed by a confocal laser scanning microscope (CLSM). The fluorescence image obtained from **starEO-*b*-PNIPAM** (20 mg) in an aqueous pyrene solution (6×10^{-7} M, 10 mL) exhibited a green emission resulting from the pyrene transferred into the micelles (Figure 2b).

It has been well known for many years that the solubility of certain proteins in water decreases in the presence of a salt. Similar salt effects on the solubility of PNIPAM in an aqueous solution have been also reported. The LCST of PNIPAM was found to decrease continuously as the concentration of a salt increased. It is generally believed that the salt ions interact with water molecules surrounding PNIPAM, leading to destabilize the hydrogen bondings between the polymer and water.[25-27] As a result, the PNIPAM-PNIPAM interactions become stronger than the water-PNIPAM interactions and the polymer molecules precipitate. We investigated the effects of sodium chloride and potassium chloride on the LCST and micelle formation of **starEO-*b*-NIPAM**. Aqueous solutions of **P2** containing various amounts of the salts were prepared and their cloud point temperatures were measured. The LCST of **P2** decreased sharply as the concentration of sodium chloride increased, while decreased slowly with potassium chloride (Figure 4). For comparison, we also prepared linear **PEG-*b*-PNIPAM** (M_n = 7100, PDI = 1.08) using linear macroinitiator **5** obtained from PEG with M_n of 750. In contrast to **P2**, the

linear block copolymer showed a continuous decrease in the LCST according to the concentrations of both sodium and potassium chloride salts.

P2 has a cyclotriphosphazene head with five methoxyethoxyethoxy groups. This cyclotriphosphazene polypodand is known to have better complex forming capability and selectivity for metal cations than linear PEG.[28,29] We presume that methoxyethoxyethoxy group substituted cyclotriphosphazene polypodand would interact with potassium ions more strongly and disturb their interaction with PNIPAM.

In summary, we prepared thermosensitive double hydrophilic block copolymers (**starEO-*b*-NIPAM**) by ATRP of NIPAM using a five oligo(ethylene oxide) substituted cyclotriphosphazene as an initiator. They were soluble in water below the LCSTs and became amphiphilic to form micelles above the LCSTs. The water solubility of the polymers decreased in the presence of a salt and the effect of potassium chloride on the LCST was less pronounced compared with sodium chloride.

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