



Supporting Information

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Comparison of thermosensitive properties between poly(amidoamine) dendrimers having peripheral N-isopropylamide groups and linear polymers with the same groups

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Experimental

Materials

PAMAM (Starburst) G5 and G4.5 dendrimers and PAMAM-OH G5 dendrimer were purchased from Aldrich Chemical (Milwaukee, WI). N-Hydroxysuccinimide (HO-NSu) N,N'-dicyclohexylcarbodiimide (DCC), and glutaric anhydride was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Isopropylamine, succinic anhydride, triethylamine (TEA), urea, N, N-dimethyl formamide (DMF), and Dimethyl sulfoxide (DMSO) were supplied from Kishida Chemical (Osaka, Japan).

Synthesis of NIPAM-0-G4.5 dendrimer

HO-NSu (1.4 mmol), DCC (1.5 mmol) were added to PAMAM-G4.5 (1.1 mmol) dissolved in DMSO (3 ml), and stirred for 4h. Then, isopropylamine and triethylamine (TEA) (1.7 mmol) were added to the solution and stirred for 5 days at room temperature. The resultant dendrimer having NIPAM groups (NIPAM-G5) was recovered and purified using Sephadex LH-20 column with methanol as the eluent. The obtained dendrimer was further purified by dialysis against distilled water. Yield of NIPAM-G5 was 66 %.

NIPAM-G5; ^1H NMR (D_2O); δ 1.12, 1.14 (d), 2.44 (br), 2.66 (br), 2.85 (br), 3.32 (br), 3.90 (br). ^{13}C NMR (D_2O); δ 24.4, 35.7, 38.0, 39.5, 44.5, 52.0, 54.2, 176.0, 177.2.

Synthesis of N-isopropylsuccinamic acid

An 8M solution of isopropylamine in ethanol (5.5ml, 44 mmol) was added to an ice-cooled solution of succinic anhydride (2.0g, 20 mmol) in ethanol (10ml) and the reaction mixture was stirred at 0°C for 90 min and then for 1h at RT, and the solvent was evaporated. The residue was dissolved in ethyl acetate (50ml), washed with 1 M HCl (40 ml) and then dried over MgSO_4 . Evaporation of the solvent gave the crude product. Subsequent purification by recrystallization in THF/diethyl ether yielded N-isopropylsuccinamic acid. Yield of N-isopropyl succinamic acid was 57.8 %.

^1H NMR (DMSO-d_6); δ 1.02, 1.03 (d), 2.26 (t), 2.40 (t), 2.50 (m), 3.79 (m), 7.68 (br).

Synthesis of 4-isopropylcarbamoyl butyric acid

An 8M solution of isopropylamine in ethanol (5.5ml, 44 mmol) was added to an ice-cooled solution of succinic anhydride (2.28g, 20 mmol) in ethanol (10ml) and the reaction mixture was stirred at 0°C for 90 min and then for 1h at RT, and the solvent was evaporated. The residue was dissolved in ethyl acetate (50ml), washed with 1 M HCl (40 ml) and then dried over MgSO_4 . Evaporation of the solvent gave the crude product. Subsequent purification by recrystallization in THF/diethyl ether yielded 4-isopropylcarbamoyl butyric acid. Yield of 4-isopropylcarbamoyl butyric acid was 62.3 %.

^1H NMR (DMSO-d_6); δ 1.00, 1.02 (d), 1.68 (m), 2.03 (t), 2.17 (t), 3.79 (m), 7.65 (br).

Synthesis of NIPAM-3-G5 dendrimer

HO-NSu (1.1 mmol) and DCC (1.2 mmol) were added to N-isopropylsuccinamic acid (0.9 mmol) dissolved in DMF (3 ml) at 0°C, and stirred for 4h. Then, PAMAM G5 dendrimer (4.6 μmol) dissolved in DMSO (3 ml) and TEA (1.3 mmol) were added to the solution and stirred for 5 days at room temperature. The resultant dendrimer was recovered and purified using Sephadex LH-20 column with methanol as the eluent. The obtained dendrimer was further purified by dialysis against distilled water. Yield of NIPAM-3-G5 dendrimer was 67.6%.

^1H NMR (D_2O); δ 1.12, 1.14 (d), 1.85 (m), 2.23 (m), 2.21 (br), 2.42 (br), 2.64 (br), 2.82 (br), 3.31 (br), 3.90 (m). ^{13}C NMR (D_2O); δ 24.4, 24.8, 35.5, 37.0, 38.0, 39.6, 41.5, 44.4, 52.0, 54.1, 177.0, 177.1, 177.5.

Synthesis of NIPAM-4-G5 dendrimer

HO-NSu (1.3 mmol) and DCC (1.4 mmol) were added to 4-isopropylcarbamoyl butyric acid (1.1 mmol) dissolved in DMF (3 ml) at 0°C, and stirred for 4h. Then, PAMAM G5 dendrimer (5.6 μmol) dissolved in DMSO (3 ml) and TEA (1.6

mmol) were added to the solution and stirred for 5 days at room temperature. The resultant dendrimer was recovered and purified using Sephadex LH-20 column with methanol as the eluent. The obtained dendrimer was further purified by dialysis against distilled water. Yield of NIPAM-4-G5 dendrimer was 72.9%.

^1H NMR (D_2O); δ 1.12, 1.14 (d), 1.85 (m), 2.23 (m), 2.21 (br), 2.42 (br), 2.64 (br), 2.82 (br), 3.31 (br), 3.90 (m). ^{13}C NMR (D_2O); δ 24.4, 24.8, 35.5, 37.0, 38.0, 39.6, 41.5, 44.4, 52.0, 54.1, 177.0, 177.1, 177.5.

Syntheses of poly(NIPAAm) and poly(NIPAAm-co-AAm)

Poly(NIPAAm) and poly(NIPAAm-co-AAm) were prepared by free-radical polymerization using AIBN as the initiator. NIPAAm and AAm in the ratio of 10/0 or 9/1 (total 10 mmol) and AIBN (0.03 mmol) were dissolved in freshly distilled tetrahydrofuran (10 mL), and then the solution was heated to 60 °C for 6 h in N_2 atmosphere. The polymer was recovered by precipitation with diethyl ether and dried under vacuum. The polymer was dissolved in water, dialyzed against distilled water, and then recovered by lyophilization. Composition of polymers was estimated by ^1H NMR. The number average molecular weight and the weight average molecular weight of poly(NIPAAm) were estimated to be 4700 and 10000, respectively, by GPC on a Shodex KD-803 columns (Showa Denko) using *N,N*-dimethylformamide as an eluent and poly(ethylene glycol) as the standard. The number average molecular weight and the weight average molecular weight of poly(NIPAAm-co-AAm) were evaluated to be 3000 and 5300, respectively, by the same manner.

Measurements

^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 400 instrument. Chemical shifts for ^1H spectra were referenced to 3-(trimethylsilyl)-propionate-2,2,3,3- d_4 . The numbers of the NIPAM groups attached to the PAMAM dendrimers were determined from the integral ratio of the signal at 2.7-2.9 ppm, which corresponds to the protons of methylenes next to the tertiary amino groups of the dendrimer, to the signal at 1.12-1.14, which corresponds to the protons of the terminal methyls in the NIPAM groups. Numbers of terminal NIPAM groups for NIPAM-0-G4.5, NIPAM-3-G5, and NIPAM-4-G5 dendrimers were evaluated using ^1H NMR to be 126.4, 127.6, and 125.0, which agree well with those of the dendrimers' chain terminals (128) (See Figure S1). In addition, ^{13}C NMR demonstrated that peaks corresponding to the carbons next to the terminal amine groups of the G5 dendrimer or the carbons of terminal carboxyl groups of the G4.5 dendrimers disappeared completely after reaction with the corresponding amine or carboxylic acids (See Figure S2). These results indicate that essentially every chain terminal of these dendrimers was combined to the corresponding terminal groups for these surface-modified dendrimers.

Turbidity of aqueous solutions of alkylamide-terminated dendrimers was measured using a Jasco Model V-560 spectrophotometer equipped with a peltier type thermostatic cell holder coupled with a controller ETC-505T. The heating rate of sample cells was adjusted at 1.0 °C min^{-1} . The lower critical solution temperatures were taken as the initial break points in the resulting transmittance versus temperature curves.

Differential scanning calorimetry (DSC) was performed with a DSC 120 microcalorimeter (Seiko Electronics). The heating rate was 0.5 °C/min.

Emission spectra of PyCHO were obtained on a Jasco Model V-560 spectrophotometer equipped with a peltier type thermostatic cell holder coupled with a controller ETC-273T with excitation at 365.5 nm using slit widths of 5 nm.

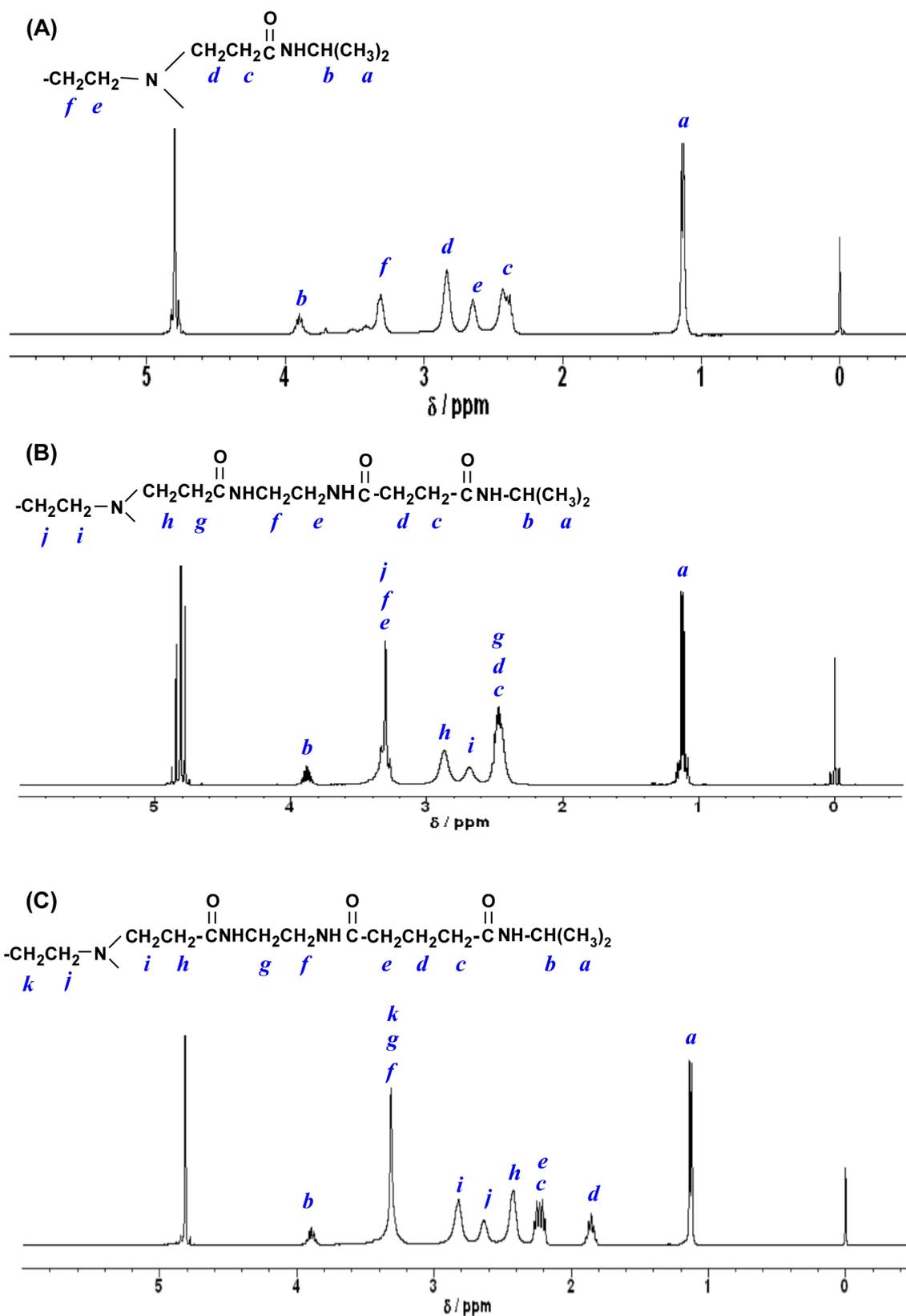
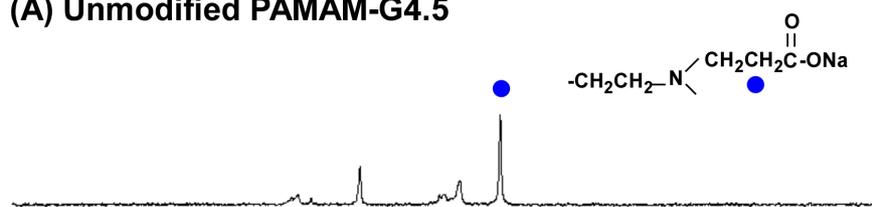
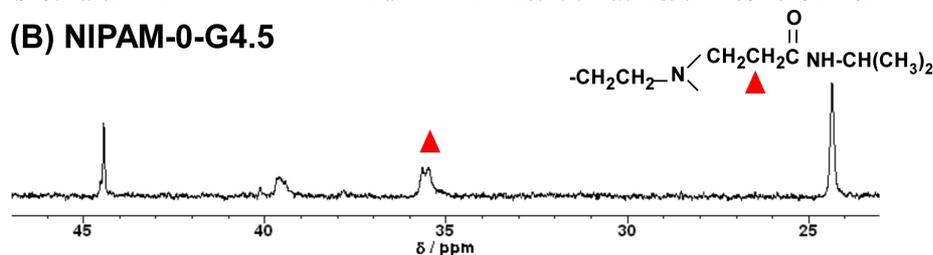


Figure S1. ^1H NMR spectra of NIPAM-0-G4.5 (A), NIPAM-3-G5 (B), and NIPAM-4-G5 (C) dendrimer in D_2O

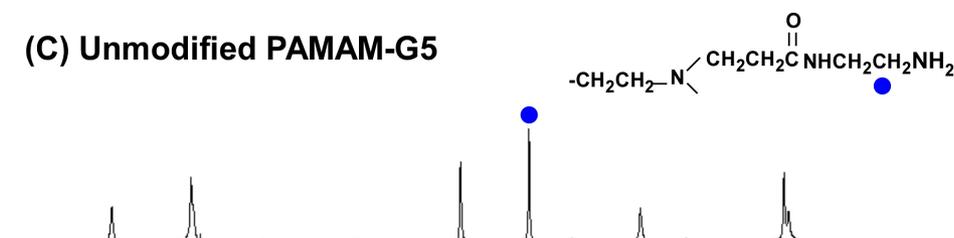
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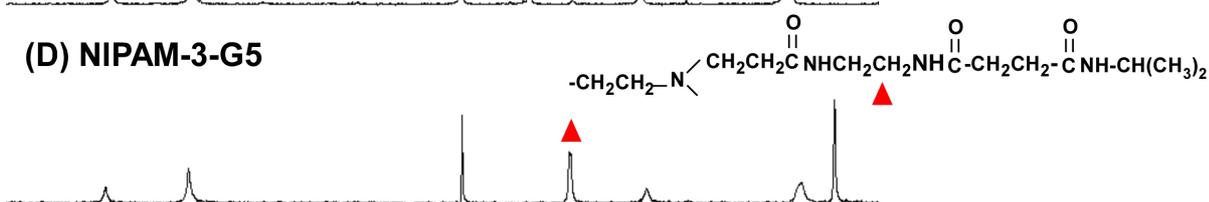
(B) NIPAM-0-G4.5



(C) Unmodified PAMAM-G5



(D) NIPAM-3-G5



(E) NIPAM-4-G5

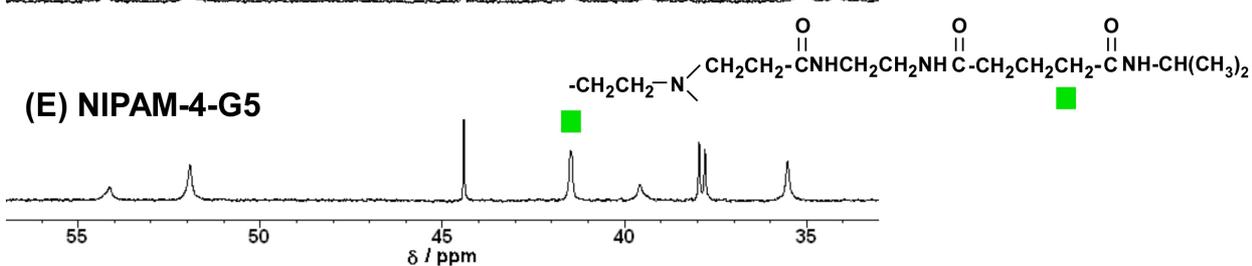


Figure S2. ^{13}C NMR spectra of PAMAM G4.5 (A), NIPAM-0G4.5 (B), PAMAM-G5 (C), NIPAM-3-G5 (D), and NIPAM-4-G5 (E) in D_2O .