Topology effects on the LCST of end-capped poly(ethylene glycol)s

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Abstract. Poly(ethylene glycol) end-capped with pentafluorophenyl group(s) in ABA (FP-PEG-FP) and AB (mPEG-FP) types were prepared. Even though they were similar in composition, the lower critical solution temperature (LCST) of FP-PEG-FP was observed at 23ºC, whereas that of mPEG-FP was observed at 65ºC. To understand the large difference in solution behaviour of the two polymers, UV-VIS spectroscopy, microcalorimetry, 1H-NMR spectroscopy, and dynamic light scattering were used. FP-PEG-FP has two hydrophobic pentafluorophenyl groups at the ends of hydrophilic PEG (1000 Daltons), whereas mPEG-PF has a highly dynamic PEG (550 Daltons) block that are anchored to a hydrophobic pentafluorophenyl group. PF-PEG-PF not only has a smaller conformational degree of freedom than mPEG-PF but also can form extensive intermolecular aggregates, therefore, PF-PEG-PF exhibits a significantly lower LCST than mPEG-PF. This paper suggests that topological control is very important in designing a temperature-sensitive polymer.

Keywords: end-capped PEG; fluorinated compound; LCST; temperature sensitive polymer

1. Introduction

Temperature sensitive polymers and their understanding of the transition mechanism have been drawing attention for last several decades (Hoffman 2014, Nishida *et al*. 2004, Jiang and Zhao 2008, Moon *et al*. 2012, Yu and Ding 2008, Zhang *et al*. 2008). In particular, temperature-sensitive coil-to-globule transition of poly(N-isopropyl acrylamide) (PNIPAAm) caused by dehydration around the amide bonds leads to the precipitation of the polymer in water at its lower critical solution temperature (LCST) of 32ºC which is in a biologically important temperature range of 20-40ºC (Heskins *et al*. 1968, Hou and Wu 2014). Based on its aqueous solution behaviour, homo- and copolymers of N-isopropyl acrylamide have been extensively investigated as stimuli-sensitive actuators, microgels, cell-sheet engineering materials, and nanocatalysts (Nishida *et al*. 2004, Pelah *et al*. 2007, Brugger and Ritchering 2008, Choi *et al*. 2005). LCST of a temperature-sensitive polymer can be controlled by molecular weight and composition of the polymer, or by adding salts to the polymer aqueous solution (Zhang *et al*. 2007, Hiruta *et al*. 2014, López-León *et al*. 2014, Yin *et al*. 2006).

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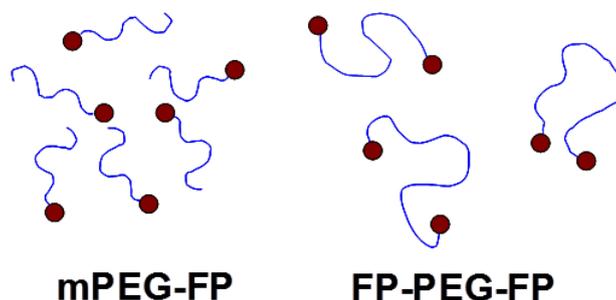*Jin Young Kim, Hyo Jung Moon, Du Young Ko and Byeongmoon Jeong*

Fig. 1 Schematic presentation of fluorohydrocarbon end-capped poly(ethylene glycol)s

The search for a new temperature sensitive polymer with a specific structure is a still hot issue. Here, we are reporting a temperature-sensitive poly(ethylene glycol) end-capped by pentafluorophenyl group, and the transition temperature could be modulated by a simple topological variation at a fixed composition. End-capping is a simple and efficient method in modulating temperature-sensitivity of polymers (Yu *et al*. 2006, Chang *et al*. 2009, Kim *et al*. 2009). We compared solution behaviour of monomethoxypoly(ethylene glycol)-pentafluorophenylurethane (mPEG-FP; CH_3O-$(CH_2CH_2O)_{11.8}$-$CONH$-C_6F_5) and pentafluorophenylurethane-poly(ethylene glycol)-pentafluorophenylurethane (FP-PEG-FP; C_6F_5-$CONH$-$O(CH_2CH_2O)_{22.7}$-$CONH$-C_6F_5) that have a similar composition, however a different topology (Fig. 1).

2. Materials and methods

2.1 Materials

mPEG (MW=550 Daltons, Aldrich), PEG (MW=1000 Daltons, Aldrich), and pentafluorophenylisocyanate were used as received. Toluene (J.T. Baker) was distilled over sodium before use.

2.2 Synthesis

To prepare mPEG-PF, mPEG (4.0 g, 7.27 mmole; MW 550 Daltons; Aldrich) was dissolved in toluene (100 mL) and the residual water was removed by azeotropic distillation to a final volume of about 20 mL. Pentafluorphenylisocyanate (1.14 g, 8.72 mmole) was added to the reaction mixtures. They were stirred at room temperature for 24 hours. After removing the solvent, the polymer was purified by repeated dissolution in the chloroform, followed by precipitation into diethyl ether, three times. The yield was 67%. PF-PEG-PF was similarly synthesized by using PEG with dihydroxyl end groups instead of mPEG with a monohydroxyl end group. The yield was 70%.

2.3 FTIR spectroscopy

The FTIR spectra (FTIR spectrophotometer FTS-800; Varian) of polymers were investigated to

confirm the structures of mPEG-PF and PF-PEG-PF.

2.4 Gel permeation chromatography

The gel permeation chromatography system (Waters 515) with a refractive index detector (Waters 410) was used to obtain the molecular weights and molecular weight distribution of polymers. *N,N*-dimethyl form amide was used as an eluting solvent. Poly(ethylene glycol)s with a molecular weight range of 400-20,000 Daltons were used as the molecular weight standards. An OHPAK SB-803QH column (Shodex) was used.

2.5 Turbidity

Absorbance (Scinco S-3100) of polymer aqueous solutions (10.0 wt.%) were measured at 550 nm as a function of temperature in a range of 5℃-75℃. The solution temperature was equilibrated for 20 minutes at each temperature.

2.6 Microcalorimetry

A differential scanning calorimeter (Microcal, VP- DSC) was used to study heat exchanges of the polymer aqueous solutions (10.0 wt.%, 0.51 mL) in a temperature range of 5℃-90℃ with a heating rate of 1.0℃/min.

2.7 ^{1}H-NMR spectra

^{1}H-NMR spectral changes of the mPEG-FP and FP-PEG-FP (10.0 wt.% in D_2O) were investigated as a function of temperature in a range of 5℃-75℃. The solution temperature was equilibrated for 20 minutes at each temperature.

2.8 Dynamic light scattering

The apparent size of polymer or polymer aggregates in water (10.0 wt.%) was studied by a dynamic light scattering instrument (ALV 5000-60-0) as a function of temperature. A YAG DPSS-200 laser (Langen, Germany) operating at 532 nm was used as a light source. Measurements of the scattered light were made at an angle of 90℃ to the incident beam. The results of dynamic light scattering were analyzed by the regularized CONTIN method.

3. Results and discussion

To prepare the pentafluorophenyurethane end-capped poly(ethylene glycol), residual water of PEG was removed by azeotropic distillation from anhydrous toluene. Then, excess amount of pentafluorophenyisocyanate was added. FTIR spectra and gel permeation chromatogram show the quantitative conversion of the end-capping reaction. An O-H stretching band of PEG at 3400 cm^{-1}-3600 cm^{-1} disappeared and a urethane carbonyl stretching band of pentafluorophenyurethane end-capped poly(ethylene glycol) at 1740 cm^{-1} appeared (Fig. 2(a)). The end-capped polymer has a larger hydrodynamic volume in *N,N*-dimethyl form amide than unmodified PEG or mPEG.
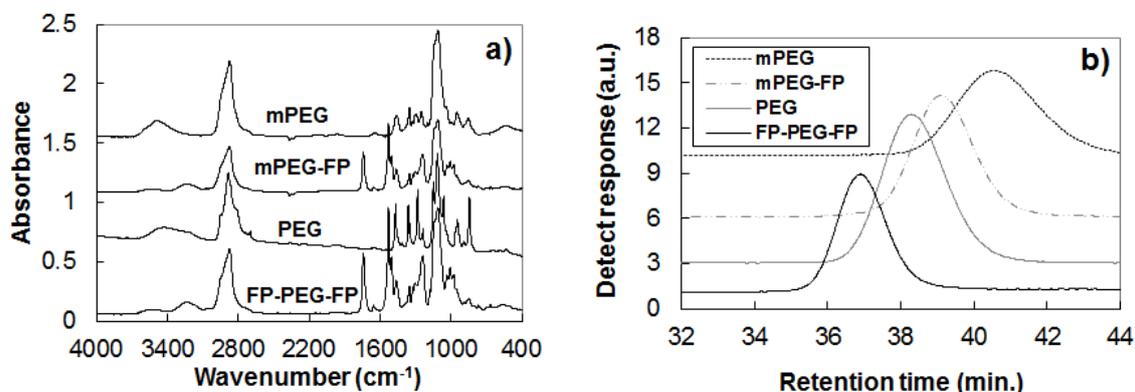
Fig. 2 FTIR (a) and gel permeation chromatograms (b) of mPEG (550 Daltons), mPEG-FP, PEG (1000 Daltons), and FP-PEG-FP

Therefore, retention time of the end-capped polymer was shortened by 2-3 minutes than the unmodified polymers. The unimodal distribution of the end-capped polymers indicated that the polymers were sufficiently purified to discuss the physicochemical properties (Fig. 2(b)).

In order to measure the temperature sensitive solution behaviour, turbidity of the polymer aqueous solutions (10 wt.%) was investigated by the UV-VIS spectrophotometer at 550 nm. The clear-to-turbid transition of mPEG-FP aqueous solution was observed at 65°C, whereas that of FP-PEG-FP aqueous solution was observed at 23°C, even though both polymers had a similar composition (Fig. 3(a)). mPEG aqueous solution did not undergo such a transition in a temperature range of 5°C-75°C. The clear-to-turbid transition of the polymers is an endothermic process as shown by the microcalorimetric thermograms of the polymer aqueous solutions (Fig. 3(b)), suggesting that the transition is an entropy driven process. The enthalpy of transition (ΔH_o) was calculated to be 3.74 cal/g of polymer or 2.83 kcal/mol of polymer (by two point calibration between 55°C-80°C) and 0.82 cal/g of polymer or 1.16 kcal/mol (by two point calibration between 20°C-35°C) for mPEG-FP solution (10 wt.%) and FP-PEG-FP aqueous solution (10 wt.%), respectively. In case of PNIPAAm aqueous solution measured from 5.0 wt.%, ΔH_o=13.9 cal/g of polymer (Vernon *et al*. 2000).

To investigate temperature-sensitive transition mechanism of the polymer, ^{1}H-NMR spectra of the polymer aqueous solutions (10 wt.% in D_2O) were studied as a function of temperature. Broadening of the PEG peak at 3.7-3.8 ppm and development of new peaks at 3.6-3.7 ppm were observe as the temperature increased above the transition temperature. Dehydration of PEG was suggested for such a behavior for PEG and PEG containing block copolymers (Chen *et al*. 2014, Ko *et al*. 2013, Singh *et al*. 2015, Park *et al*. 2010, Li *et al*. 2014). In particular, the new peaks at 3.6-3.7 ppm were apparent for FP-PEG-FP at above its clear-to-turbid transition temperature, suggesting that the highly dehydrated PEGs experienced new magnetochemical environments. Interestingly, the water peak at 5.0 ppm was steadily downfield-shifted, whereas chemical shift of the NH peak of connecting urethane at 4.3-4.4 ppm did not change (Fig. 4). When a hydrophobe is located in water, water molecules tend to form a shell (Creighton 1993). During the clear-to-turbid transition, water molecules are released from tight shell around the hydrophobe to bulk water and hydrophobic interactions increase. Therefore, the water peak appeared in the upfield of the ^{1}H-NMR spectra of the end-capped polymers at high temperature. ^{1}H-NMR spectra suggest that the

LCST behavior of the hydrophobically end-capped PEG is caused by the dehydration of PEG. In addition, the hydrophobic capping molecule reduces the conformational degree of freedom of PEG and reduces the LCST in the entropically driven process.

Dynamic light scattering of the polymer aqueous solution provides information on the change in apparent size of a polymer and their aggregates. As the temperature increases, apparent size of mPEG-FP aggregates in water changed from hundreds nm to 2-5 nm, and to thousands nm (Fig. 5(a)). The particles with hundreds nm in size was observed only for mPEG-FP at low temperature (5-15°C). This behavior is related to the intermolecular aggregates because the concentration of polymers (10 wt.%) was higher than C* (critical overlap concentration at which polymer contours just touch each other). At a low concentration of 1.0 wt.%, such a large particle was not observed (Supporting Information: Fig. S1). FP-PEG-FP can adapt a more shrunken conformation around the two FPs and a particle size of 2 nm-5 nm was observed in a temperature range of 5°C-15°C (Fig. 5(b)). As the temperature increase to clear-to-turbid transition temperature of 23°C, aggregates with thousands nm in size were observed due to the extensive aggregation of the polymers.
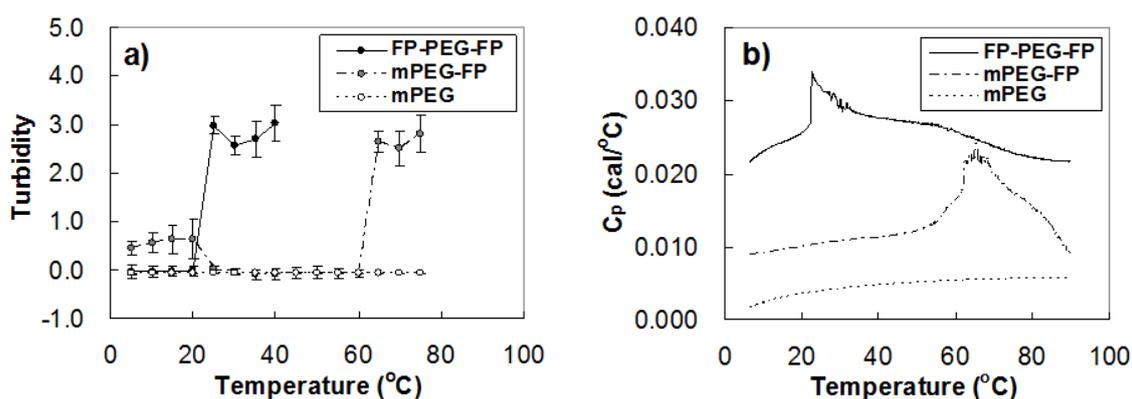

Fig. 3(a) Turbidity of mPEG, mPEG-FP, FP-PEG-PF as a function of temperature at 10.0 wt.%. The turbidity was defined as -Log (transmittance) at 550 nm (b) Microcalorimetric measurement of mPEG, mPEG-FP, FP-PEG-PF at 10.0 wt.%
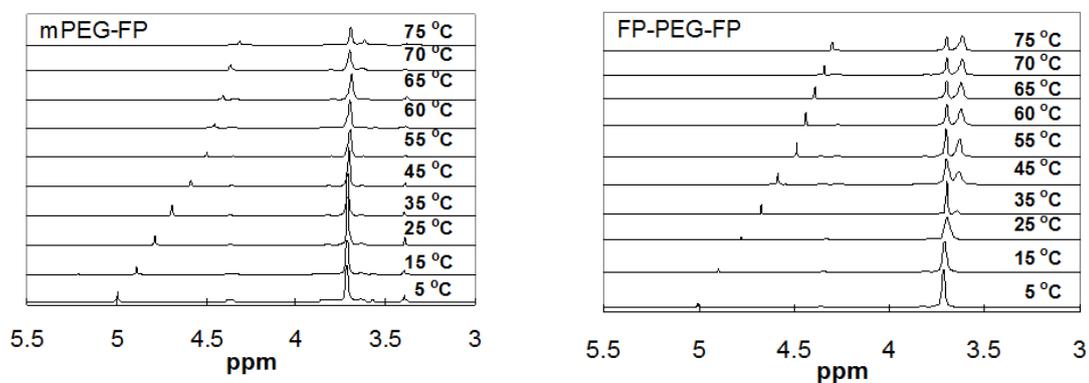

Fig. 4 ^{1}H-NMR spectra (in D_2O) of mPEG-FP and FP-PEG-PF as a function of temperature at 10.0 wt.%
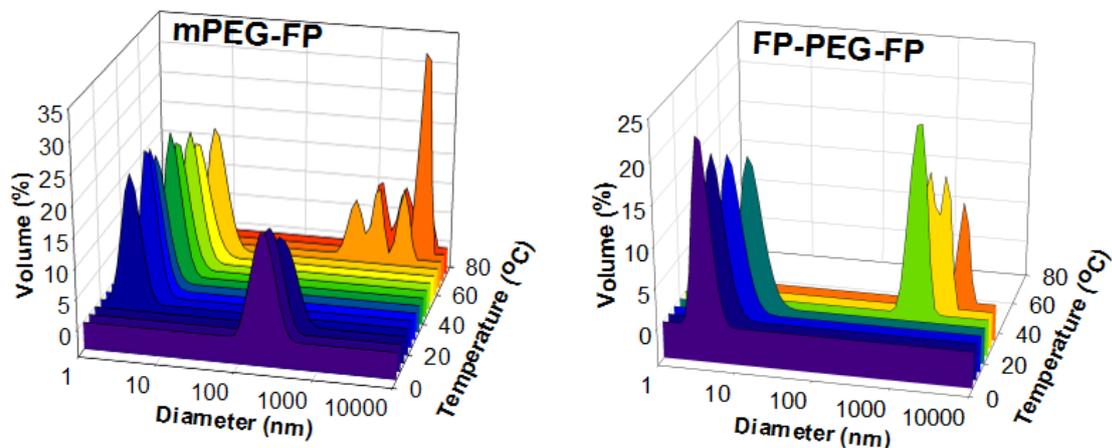
Fig. 5 Apparent size of mPEG, mPEG-FP, FP-PEG-PF at 10.0 wt.% determined by dynamic light scattering

Fluorinated compounds have been applied as biomaterials for their chemical and biochemical inertness, low surface tension, and unique lipophilicity (Wang *et al.* 2014, Yoshimura *et al.* 2006, Busschaert *et al.* 2011). In addition, fluorinated compounds have been used as a plasma extender for their high oxygen solubility and blood compatibility (Wijekoon *et al.* 2013, Tan *et al.* 2012). Considering the biomedical applications, the current temperature-sensitive polymers can be applied for biosensor, drug delivery, and coating for cell culture plates with tunable surfaces similar to PNIPAAm (Choi *et al.* 2014, Jenkins *et al.* 2015, Barata *et al.* 2015, Nishinaga *et al.* 2011, Nishida *et al.* 2004).

4. Conclusions

LCST of a polymer aqueous solution is a function of balance between hydrophobicity and hydrophilicity of the polymer. However, a large difference in LCST was observed for mPEG-PF and PF-PEG-PF with a similar composition, and thus a similar hydrophobicity scale. UV-VIS spectroscopy, microcalorimetry, ^{1}H-NMR spectroscopy, and dynamic light scattering of the polymer aqueous solutions suggested the entropically driven dehydration of PEG was the main mechanism of the LCST of the mPEG-FP and FP-PEG-FP. And about 4 times of energy are required for the transition of mPEG-PF as compared with PF-PEG-PF at the same concentration. Compared with mPEG-PF, PF-PEG-PF has a smaller conformational degree of freedom in water and a high potential of intermolecular aggregation among the hydrophobic moieties. This paper suggests that topological control as well as hydrophobicity of a molecule plays an important role in determining stimuli-sensitive behavior of the polymer.

Acknowledgments

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