Synthesis and Solubility of (Mono-) End-Functionalized Poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) Graft Copolymers with Varying Macromolecular Architectures

Dong Zhang, Celia Macias, and Christine Ortiz*

Department of Materials Science and Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139

Received April 5, 2004
Revised Manuscript Received June 21, 2004

Introduction

Comblike, side-chain graft copolymers, also called molecular bottle brushes, are branched macromolecules composed of an array of side chains attached to a main chain. Such a motif exists in biology; for example, aggrecan macromonomers which provide the compressive stiffness of cartilage tissue, have a protein core backbone and chondroitin sulfate glycosaminoglycan polysaccharide side chains spaced 2−4 nm apart.[1] Recently, there has been a large interest in theoretically studying such systems,[2] as well as preparing analogous synthetic macromolecules[3] due to their ability to exhibit a broad array of unique conformations[4] and nanomechanical properties[5,6] including, in many cases, stimulus responsiveness.[7] Property control can be achieved via the choice of side chain and main chain chemical structures, as well as the macromolecular architecture (e.g., side chain graft density, length, and distribution along the main chain) to tailor entropic, excluded volume, electrostatic, and other noncovalent interactions. Precise design for particular applications will require the synthesis of well-defined structural libraries in conjunction with theoretical and experimental studies of intra- and intermolecular interactions, thus facilitating a detailed understanding of fundamental nanoscale structure−property relationships.

Recently, we reported the synthesis, characterization, and single macromolecule elasticity measurements of a neutral (mono-) end-functionalized graft copolymer, thiol-terminated poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) or SH-poly(HEMA-*g*-EG) with a 1% PEG (poly(ethylene glycol)) graft density[6] via the atom transfer radical polymerization (ATRP) method[8] with protecting group chemistry.[9] (Mono-) end-functionalization provides the ability to chemically end-graft these polymers to surfaces[6] and small structures (e.g., nanoparticles, nanofibers, etc.) in a well-defined manner. Expanding upon this methodology, the first goal of this research was to synthesize a library of water-soluble SH-poly(HEMA-*g*-EG) graft copolymers with varying macromolecular architecture; i.e., PEG and PHEMA (poly(2-hydroxyethyl methacrylate)) molecular weights, EG/HEMA mole ratios, and PEG side chain graft density. PEG and PHEMA were chosen as the model systems since they are each individually known to exhibit unique interactions in aqueous environments.[10,11] For example, PEG exhibits a hydrogen-bonded, water-bound, contracted, helical trans−trans−gauche local conformation,[12] and PHEMA has been suggested to have a significant amount of bound structured water.[13] Hence, both are technologically important materials used extensively for biomedical engineering applications, since such unique and strong interactions with water are likely to be a major contributor to bioinertness.[14] Aside from the potential to create surfaces with improved biocompatibility, PEG/PHEMA graft copolymers also have potential for applications in areas such as single macromolecule nanomechanical design (control of molecular elasticity) and as nanoreactors for nanoparticle preparation.[6,15−20] The second goal of this study was to establish the relationship between macromolecular architecture and composition with aqueous solubility, as quantified by the cloud point temperature in dilute solution, thus providing insight into the nature of macromolecular intra- and intermolecular interactions.

Experimental Section

Synthesis and Characterization. SH-poly(HEMA-*g*-EG) graft copolymers were prepared in a manner similar to that described previously[6] by ATRP using CuBr/2-(2,4-dinitrophenylthio)ethyl 2-bromo-2-methylpropionate initiator with the following modifications. For the synthesis of the SH-poly(HEMA-*g*-EG)$_{106K}$, SH-poly(HEMA-*g*-EG)$_{87K}$, and SH-PHEMA$_{61K}$ 34 g (0.016 mol), 64 g (0.031 mol), and 0 g (0 mol) of PEG$_{2080}$ were employed, respectively. The numerical subscript in the abbreviated polymer name label refers to the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000, as determined by ^{1}H nuclear magnetic resonance (NMR). For the synthesis of the SH-poly(HEMA-*g*-EG)$_{142K}$, PEG$_{1100}$ (34 g, 0.031 mol) was used and a reaction solvent of water (34 g, 1.9 mol). For the synthesis of the SH-poly(HEMA-*g*-EG)$_{122K}$ and SH-poly(HEMA-*g*-EG)$_{54K}$, PEG$_{475}$ (15 g, 0.032 mol and 30 g, 0.063 mol, respectively) was used with the reaction solvent being water (12.5 g, 0.69 mol). The 2,4-dinitrophenyl protecting end group was removed at the end of the reaction using 2-mercaptoethanol yielding the thiol-terminated form of the polymer, as described previously.[6] Characterization was accomplished via ^{1}H NMR using a Varian Inova-501 instrument in methanol-d_4 solvents. Chemical shifts (δ) were reported in ppm concentrations downfield from an internal tetramethylsilane reference.

Cloud Point Temperature Measurements. Each polymer (1 mg) was dissolved in 1 mL of phosphate buffered saline solution with an ionic strength of 0.15 M and pH 7.4 and was stirred continuously with a magnetic stirrer for up to 5 h at room temperature. A home-built cloud point apparatus was employed for assessing solubility. A 670 nm 1 mW handheld laser pointer (elliptical spot shape of ∼1 mm × 2 mm) was shone through the solution onto a Newport photodiode. When the sample was clear, the detector produced ∼1.5 μA of current which was amplified by a custom built transconductance amplifier (Analog Devices model AD549L FET input operational amplifier with a 1 MΩ feedback resistor, bandwidth is dc to 100 kHz) to a level of 1.5 V. The diode current preamp output voltage was measured by a 6.5 digit resolution HP model 3455A DMM on the 10 V range (readout noise ∼0.01%). The resolution of the voltmeter was much greater than the laser diode power level noise limit which was ∼0.1%. The voltmeter averages the laser noise due to its rate of ∼2 readings per second. The cuvette sample temperature control chamber was an Al block mounted to two 50 W thermoelectric heater/cooler modules on each side. A Newport CNi16D44C24 was used to program the cuvette temperature. The temperature was monitored by an HP 3455A DMM using a type "k" thermocouple probe (noise ∼0.005 °C and accuracy was ∼1 °) and battery powered amplifier made by John Fluke. The

* Corresponding author. E-mail: cortiz@mit.edu.

10.1021/ma049330k CCC: $30.25
Published on Web 02/18/2005

Table 1. Characterization of Thiol-Terminated Poly(2-hydroxyethyl methacrylate) (SH-PHEMA$_{61K}$) and Poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) (SH-poly(HEMA-*g*-EG)) Graft Copolymers Synthesized in This Study and Reported Previously[6] by ^{1}H Nuclear Magnetic Resonance (NMR)

polymer[a]	MW PEG	M_n (kg/mol) NMR	PEG (%) NMR	N_{PEG} NMR	$DP_{n,EG}$ NMR	$DP_{n,HEMA}$ NMR	$DP_{n,EG}/DP_{n,HEMA}$	d_{PEG}	$L_{contour}$ PHEMA (nm)	$L_{contour}$ PEG (nm)	CP (°C)
SH-PHEMA$_{61K}$	0	61	0	0	0	469	0	0	144	0	I
SH-poly(HEMA-*g*-EG)$_{120K}$[6]	2080	120	1	8	360	794	0.4	27.2	245	20	I
SH-poly(HEMA-*g*-EG)$_{16K}$[6]	2080	16	1	1	45	111	0.4	17.0	34	20	I
SH-poly(HEMA-*g*-EG)$_{122K}$	475	122	14	87	680	621	1.1	2.2	191	5	45
SH-poly(HEMA-*g*-EG)$_{54K}$	475	54	23	52	407	226	1.8	1.3	70	5	62
SH-poly(HEMA-*g*-EG)$_{142K}$	1100	142	16	74	1630	464	3.5	1.9	143	11	>70
SH-poly(HEMA-*g*-EG)$_{106K}$	2080	106	4	20	886	497	1.8	7.3	153	20	~67
SH-poly(HEMA-*g*-EG)$_{87K}$	2080	87	10	26	1152	257	4.5	2.9	79	20	>70

[a] The numerical subscript in the abbreviated polymer name labels refer to the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000, MW_{PEG} is the known molecular weight of each PEG chain, PEG % is the PEG graft density which is defined as $N_{PEG}/DP_{n,HEMA}$, N_{PEG} is the average number of PEG chains per PHEMA chain, DP_n is the number-average degree of polymerization, d_{PEG} is the average HEMA contour length between neighboring PEG chains, $L_{contour}$ is the average contour length calculated from the known molecular weights, and CP is the cloud point temperature.

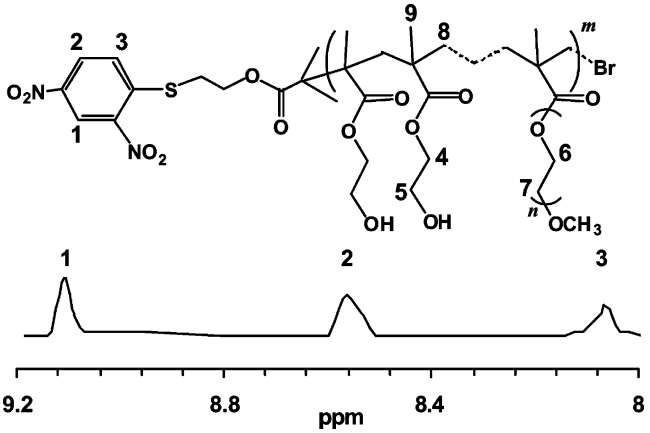


Figure 1. Chemical structure (top) and ^{1}H nuclear magnetic resonance (NMR) spectra in methanol-d_4 (bottom) showing the characteristic 2,4-dinitrophenyl protecting end group peaks between δ = 7.8−9.2 ppm for thiol-protected poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) or poly(HEMA-*g*-EG)$_{54K}$ where the numerical subscript in the abbreviated polymer name labels refer to the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000. The peak numbers labeled on the ^{1}H NMR spectrum correspond to the protons labeled in the chemical structure.

output of the temperature preamp module was connected to the input of the HP DMM. The heating rate was 0.1 °C/3 s and the range was 40−67 °C. A Kepko 50 V at 8 A bipolar power amplifier boosted the analogue output voltage from the temperature controller to the 24 V at 4 A maximum level required by the two TE modules wired in series.

Results and Discussion

Synthesis and Characterization. As summarized in Table 1 (column 2), the molecular weight of the PEG side chains employed was between 0 (PHEMA homopolymer) and 2080 g/mol. The success of the synthesis of the graft copolymers with varying macromolecular architecture was verified by ^{1}H NMR (methanol-d_4). The presence of the 2,4-dinitrophenyl protecting end group was confirmed for all of the graft copolymers by peaks present at δ 9.1, 8.5, and 8.1 ppm corresponding to the protons at positions 1, 2, and 3 shown in the chemical structure of Figure 1, which also shows an example of the ^{1}H NMR data in this ppm region for the thiol-protected poly(HEMA-*g*-EG)$_{54K}$ graft copolymer. Treatment with mercaptoethanol resulted in the disappearance of these peaks, indicating the removal of the protecting group, while the rest of the peaks in the spectra (described following) remained unchanged. The main chain and side chain structures of the graft copolymers were verified as shown in the ^{1}H NMR spectra of Figure 2a (methanol-d_4): δ = 4.0 (−CH_2−OCO), 3.8 (−CH_2−OH), 2.2−1.4 (−CH_2−C), 3.6 (−O−CH_2−CH_2−O−), 1.3−0.7 (−CH_3) ppm.

By setting the integrated area at peak 1, 2, or 3 as a unit, the integrated areas at peaks 4 or 5 give half of the number of 2-hydroxyethyl groups. Thus, the corresponding molecular weight of the HEMA portion of the backbone of the polymer could be obtained. The PEG graft density was defined as A/B = mol of PEG/mol of HEMA = $N_{PEG}/DP_{n,HEMA}$ where N_{PEG} is the average number of PEG chains per PHEMA chain and $DP_{n,HEMA}$ is the number-average degree of polymerization of the PHEMA backbone. The PEG graft density was obtained by the following equations: $A = (A_6 + A_7)/4n_{EG}$ which is the ratio of $^1/_4$ of the integrated area of peaks 6 + 7 (since peaks 6 and 7 form one overlapping peak) divided by n_{EG} which is the known number of EG monomers per PEG chain and $B = (A_4/2) = (A_5/2)$ which is half of the integrated area of peak 4 or 5. The total molecular weight was calculated from the molecular weight of the HEMA backbone and PEG graft density which yielded the total molecular weight of the polymer. The number-average molecular weight, M_n, for the graft copolymers ranged between 16 and 142 kg/mol and the ratio of the number-average degree of polymerization of EG to HEMA, $DP_{n,EG}$ / $DP_{n,HEMA}$, ranged between 0 and 4.5. The PEG side chain graft density ranged between 0% (PHEMA homopolymer) and 23%. A summary of the parameters describing the macromolecular architecture of the polymers synthesized is given in Table 1 and corresponding schematics are given in Figure 2b.

Cloud Point Temperature Measurements. The raw data from the cloud point temperature measurements of the graft copolymers are given in Figure 3a and the last column of Table 1. The SH-PHEMA$_{61K}$ ($DP_{n,HEMA}$ = 469) homopolymer was insoluble through the entire temperature range probed in the cloud point measurements (40−67 °C). While PHEMA gels are known to attain a relatively large degree of water hydration (up to ~42%)[21] at room temperature, linear PHEMA homopolymer with $DP_{n,HEMA}$ = 40 starts to exhibit insoluble fractions[18] (consistent with our data). The insolubility of higher molecular weight PHEMA is presumably due to attractive intramolecular interactions outweighing intermolecular bonding with water.

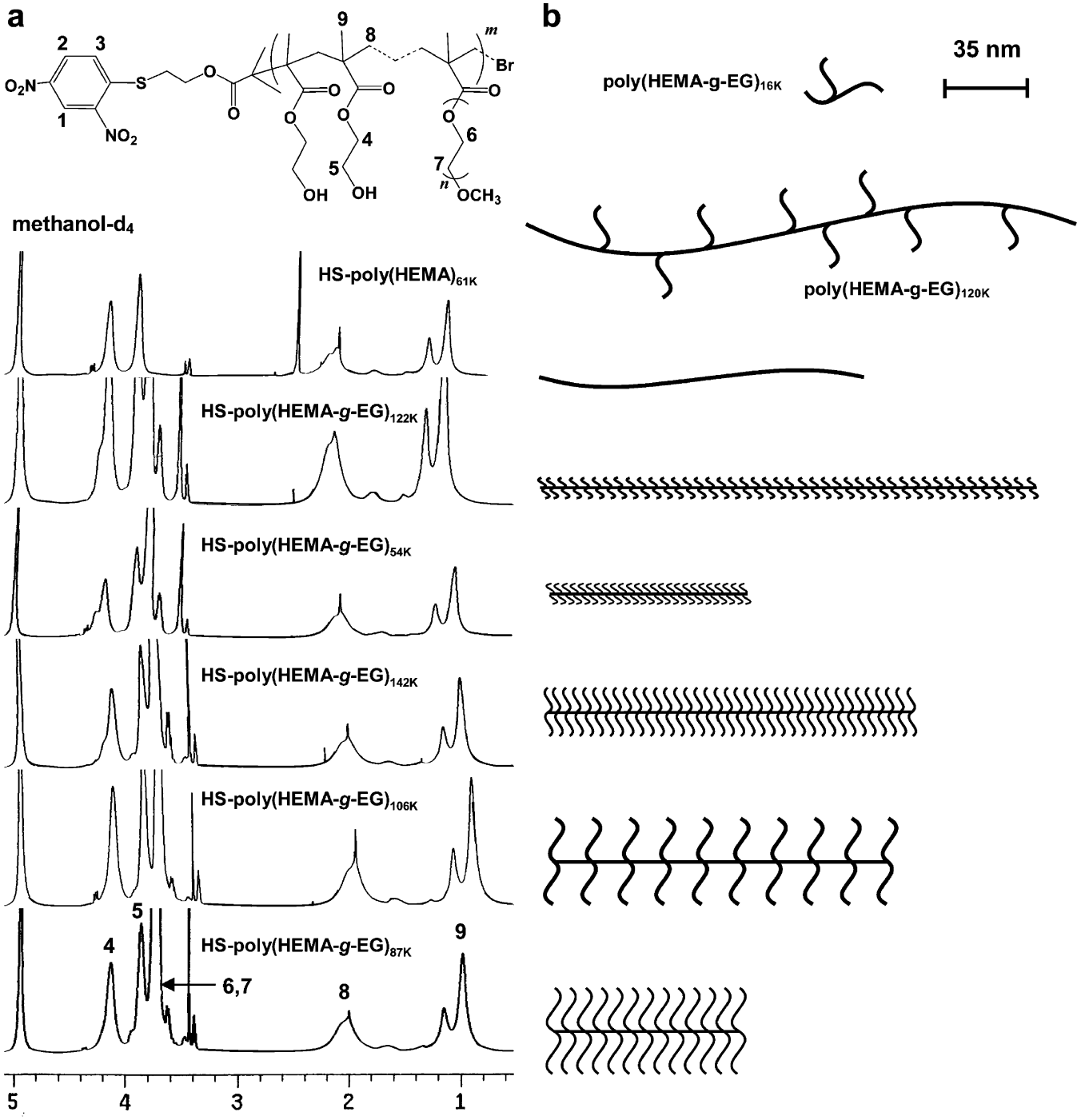
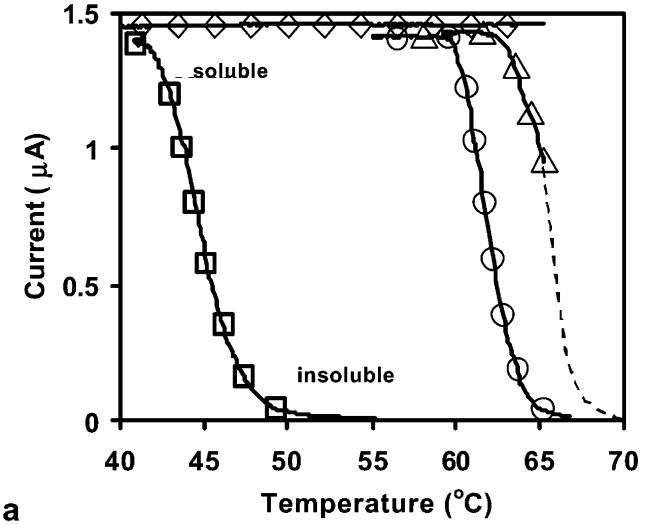


Figure 2. (a) Chemical structure (top) and ^{1}H nuclear magnetic resonance (NMR) spectra between $\delta = 0.6-5$ ppm for the thiol-protected poly(2-hydroxyethyl methacrylate) ($PHEMA_{61K}$) and poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) (poly(HEMA-*g*-EG)) graft copolymers synthesized in this study. The numerical subscript in the abbreviated polymer name labels refer to the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000. The peak numbers labeled on the lowermost NMR spectra (poly(HEMA-*g*-EG)$_{87K}$) correspond to the protons labeled in the chemical structure at the top of Figure. The ^{1}H NMR spectra for the poly(HEMA-*g*-EG)$_{120K}$ and poly(HEMA-*g*-EG)$_{16K}$ were reported previously[6] and, hence, are not shown here again. (b) Schematics of poly(HEMA-*g*-EG) graft copolymers synthesized in this study and previously[6] with contour length dimensions for HEMA backbone, PEG side chains, and PEG side chain density drawn approximately to scale (the schematics are not meant to indicate the actual spatial distribution of PEG side chains along the HEMA backbone or the conformation of the polymer chains). Each polymer schematic is positioned horizontally across from its respective ^{1}H NMR spectrum.

Possible attractive intramolecular interactions, based on the chemical structure of PHEMA, include hydrophobic interactions between $-CH_2-$ groups, dispersive van der Waals attractive interactions (it is reported to have a high refractive index of 1.512[22]), and it hydrogen bonds with itself via the $-OH$ and $-O-C=O$ groups.

The addition of PEG side chains and the specific macromolecular topology had a dramatic effect on the solubility of the graft copolymers, with solubility at room temperature in phosphate buffered saline solution observed for $DP_{n,EG}/DP_{n,HEMA} > 0.4$ and an increase in cloud point temperature (representing increased solubility) with $DP_{n,EG}/DP_{n,HEMA}$ and molecular weight of the PEG side chains for constant $DP_{n,EG}/DP_{n,HEMA}$ (Figure 3b and Table 1). The graft copolymers which were soluble at room temperature exhibited inverse temper-



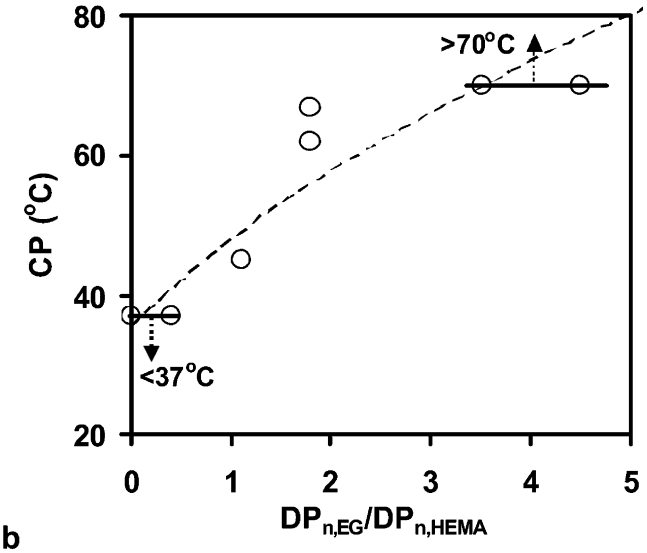


Figure 3. Cloud point temperature measurements of thiol-terminated poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) (SH-poly(HEMA-*g*-EG)) graft copolymers. (a) Raw data for (◇) Milli-Q water, (□) SH-poly(HEMA-*g*-EG)$_{122K}$ (○) SH-poly(HEMA-*g*-EG)$_{54K}$, and (△) SH-poly(HEMA-*g*-EG)$_{106K}$ in phosphate buffered saline solution. For clarity, only selected data points are shown as symbols (~200 data points were taken in total and are connected by the solid lines). For the SH-poly(HEMA-*g*-EG)$_{106K}$ graft copolymer the dashed line represents an extrapolation of the data to higher temperatures based on the functional form of the SH-poly(HEMA-*g*-EG)$_{122K}$ and SH-poly(HEMA-*g*-EG)$_{54K}$ data, since the upper temperature limit of the cloud point apparatus was 65 °C. (b) Cloud point temperatures (CP) as a function of $DP_{n,EG}/DP_{n,HEMA}$ in phosphate buffered saline solution where DP_n is the number-average degree of polymerization. The numerical subscript in the abbreviated polymer names refer to the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000. The cloud point temperature was taken at 50% of the starting current signal in the raw data plots of part a. The dashed line is an empirical best fit curve to the data to guide the eye ($y = \sqrt{A - (x - B)^2}$, $A = 271254$, $B = 520$, $R^2 = 0.85$).

ature solubility (Figure 3a), similar to lower molecular weight PHEMA (cloud point temperatures ~28−39 °C in dilute aqueous solutions at pH 6.5[18]) and PEG homopolymers.[23] For PEG, this interesting behavior has been explained in terms of entropic effects of fitting PEO molecules into the water network,[24] conformation-dependent hydrophobicity,[25] and competititive ether−water and water−water H-bonding.[26]

Conclusions

In this paper, we report the synthesis of a series of neutral SH-poly(HEMA-*g*-EG) comb-type graft copolymers with varying macromolecular architecture: $MW_{PEG} = 0-2080$ g/mol, PEG graft densities = 0−23%, $M_n = 16-142$ kg/mol, and $DP_{n,EG}/DP_{n,HEMA} = 0-4.5$. The graft copolymers were found to be insoluble in dilute aqueous solution for $DP_{n,EG}/DP_{n,HEMA} < 0.4$ and have an inverse temperature solubility with a cloud point temperature that increased (representing increased solubility) with $DP_{n,EG}/DP_{n,HEMA}$ and molecular weight of the PEG side chains for constant $DP_{n,EG}/DP_{n,HEMA}$. This study demonstrates that dramatic differences in intramolecular interactions and macromolecular properties can be obtained via variations in topology in a neutral, water-soluble system.

Acknowledgment. The authors wish to thank NSF-PECASE (0094194) and a 3M innovation fund for financial support, Professor Anne M. Mayes' group (Department of Materials Science and Engineering at MIT) for providing GPC assistance, and David Bono for cloud point apparatus construction, for measurement training, and for providing the experimental description of the apparatus.

References and Notes

(1) Ng, L. J.; Plaas, A. H. K.; Sandy, J. D.; Grodzinsky, A. J.; Ortiz, C. *J. Struct. Biol.* **2003**, *143*, 242−257.
(2) Saariaho, M.; Subbotin, A.; Szleifer, I.; Ikkala, O.; ten Brinke, G. *Macromolecules* **1999**, *32*, 4439−4443.
(3) Sheiko, S. S.; Moller, M. *Chem. Rev.* **2001**, *101*, 4099−4124.
(4) Stephan, T.; Muth, S.; Schmidt, M. *Macromolecules* **2002**, *35*, 9857−9860.
(5) Subbotin, A.; Saariaho, M.; Ikkala, O.; ten Brinke, G. *Macromolecules* **2000**, *33*, 3447−3452.
(6) Zhang, D.; Ortiz, C. *Macromolecules* **2004**, *37*, 4271−4282.
(7) Mathur, A. M.; Drescher, B.; Scranton, A. B.; Klier, J. *Nature (London)* **1998**, *392*, 367−370.
(8) Matyjaszewski, K.; Coessens, V.; Nakagawa, Y.; Xia, J.; Qiu, J.; Gaynor, S.; Coca, S.; Jasieczek, C. In *Functional Polymers: Modern Synthetic Methods and Novel Structures*; Patil, A. O., Schulz, D. N., Novak, B. M., Eds.; American Chemical Society: Washington, DC, 1998; p 16.
(9) Shaltiel, S. *Biochem. Biophys. Res. Commun.* **1967**, *29*, 178−183.
(10) Harris, J. M., Ed. *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Plenum: New York, 1992.
(11) Ghi, P. Y.; Hill, D. J. T.; Whittaker, A. K. *Biomacromolecules* **2001**, *2*, 504−510.
(12) Oesterhelt, F.; Rief, M.; Gaub, H. E. *New J. Phys.* **1999**, *1*, 6.1−6.11.
(13) Sung, Y. K.; Gregonis, D. E.; Jhon, M. S.; Andrade, J. D. *J. Appl. Polym. Sci.* **1981**, *26*, 3719−3728.
(14) Rixman, M. A.; Dean, D.; Ortiz, C. *Langmuir* **2003**, *19*, 9357−9372.
(15) Han, S.; Hagiwara, M.; Ishizone, T. *Macromolecules* **2003**, *36*, 8312−8319.
(16) Okano, T.; Uruno, M.; Sugiyama, N.; Shimada, M.; Shinohara, I.; Kataoka, K.; Sakurai, Y. *J. Biomed. Mater. Res.* **1986**, *20*, 1035−1047.
(17) Liu, S.; Weaver, J. V. M.; Save, M.; Armes, S. P. *Langmuir* **2002**, *18*, 8350−8357.
(18) Weaver, J. V. M.; Bannister, I.; Robinson, K. L.; Bories-Azeau, X.; Armes, S. P.; Smallridge, M.; McKenna, P. *Macromolecules* **2004**, *37*, 2395−2403.
(19) Save, M.; Weaver, J. V. M.; Armes, S. P.; McKenna, P. *Macromolecules* **2002**, *35*, 1152−1159.
(20) Robinson, K. L.; Khan, M. A.; de Paz Banez, M. V.; Wang, X. S.; Armes, S. P. *Macromolecules* **2001**, *34*, 3155−3158.

(21) Pinchuk, L.; Eckstein, E. C.; van der Mark, M. R. *J. Biomed. Mater. Res.* **1984**, *18*, 671−684.
(22) Seferis, J. C. In *Polymer Handbook*, 4th ed.; Brandrup, J.; Immergut, E. H.; Grulke, E. A., Eds.; Wiley-Interscience: New York, 1999; p pVI 582.
(23) Saeki, S.; Kuwahara, N.; Nakata, M.; Kaneko, M. *Polymer* **1976**, *17*, 685−689.
(24) Kjellander, R.; Florin, E. *J. Chem. Soc., Faraday Trans.* **1981**, *77*.
(25) Andersson, M.; Karlstrom, G. *J. Phys. Chem.* **1985**, *89*, 4957−4962.
(26) Dormidontova, E. E. *Macromolecules* **2002**, *35*, 987−1001.

MA049330K