Copolymers of Poly(*N*-isopropylacrylamide) Densely Grafted with Poly(ethylene oxide) as High-Performance Separation Matrix of DNA

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ABSTRACT: Two high molecular weight copolymers of poly(*N*-isopropylacrylamide) (PNIPAM) densely grafted with short poly(ethylene oxide) (PEO) chains (PNIPAM-*g*-PEO) were studied by NMR and laser light scattering. The long PNIPAM chains with densely grafted PEO branches had a random coil conformation at very dilute concentrations and low temperatures (i.e., $T \leq 30$ °C). When the temperature was increased above 31 °C, the copolymers could undergo a broad "coil-to-globule" transition. The collapsed copolymer chains had a $\langle R_g \rangle / \langle R_h \rangle$ value of about 1.0 with PNIPAM chains inside the core and the hydrophilic PEO chains on the surface. This kind of PNIPAM-*g*-PEO copolymers was studied as a DNA separation medium in capillary electrophoresis. Several advantages of the copolymers as a separation medium for DNA fragments were achieved, such as an automatic coating ability for the capillary inner wall, an easier injection into the capillary channel due to the slightly adjustable viscosity with temperature (up to 31 °C), a high resolution (i.e., one base pair resolution), and fast separation time. In contrast, the homo-PNIPAM or PEO showed worse DNA separation efficiency under similar conditions. The high DNA separation efficiency of the PNIPAM-*g*-PEO copolymers is related to the polymer chain conformation. The long copolymer chains in a random coil conformation with densely grafted PEO branches could form a physical network with a relatively stable and uniform pore size at high concentrations (i.e., ≥10 wt %). The separation medium has a high sieving ability for DNA separation in terms of DNA migration mechanisms. The collapsed copolymer chains in the globule state could destroy the chain network and thus lose the DNA separation ability.

Introduction

Capillary electrophoresis (CE) by using a polymer gel or solution as a separation medium is a powerful method in DNA separation and has been extensively used in recent years.[1] Although the pore size of cross-linked polymer gels can be controlled by the amount of monomers and cross-linked reagents, they are not suitable for large-scale DNA sequencing analysis due to the instability, irreproducibility, and difficulty in controlling the polymerization processes. Alternatively, entangled polymers have been widely used as a DNA separation medium in CE, such as liquefied agarose,[2] poly(acrylamide) (PAM),[3−5] different kinds of cellulose,[6−8] poly(ethylene oxide) (PEO),[9] and poly(dimethylacrylamide).[10] Among the various polymers, high molecular weight (M_w) PAM has achieved single-base resolution and up to 1000 bases in one run for single-strand DNA.[4] However, the PAM solution has two disadvantages: the injection is very difficult due to the very high viscosity at high concentrations, and the capillary inner wall has to be coated. One base pair (bp) resolution of double-strand DNA could also be achieved by using high-M_w PEO or a mixed polymer matrix without coating the capillary inner wall, but the solution viscosity remained too high for injection. In addition, the capillary channels had to be pretreated by a low-viscosity PEO solution before the high-viscosity matrix was injected.[11] In recent years, a viscosity-adjustable Pluronic triblock copolymer, $PEO_{99}PPO_{69}PEO_{99}$ (PPO being poly(propylene oxide)), has successively been used as a sieving matrix for DNA separation in capillary electrophoresis.[12−14] This kind of polymer has two advantages over PAM: (1) the viscosity-adjustable property of the polymer solution makes the solution easier to handle, and (2) the PEO blocks can coat the capillary inner wall automatically and thus greatly suppress electroosmosis.

There are disagreements on the mechanism of DNA separation by un-cross-linked polymer solutions. Some researchers assert that a network with a certain size of "pores" is formed in an entangled polymer solution, through which the DNA has to migrate.[15,16] This mechanism is essentially the same as that in gel electrophoresis. A recent study on DNA separation at concentrations below the entanglement threshold proposed that it is the DNA−polymer entanglements instead of simply the "pores" that leads to the separation.[17,18] The probability of entanglement of DNA with individual polymer chains at a constant concentration is proportional to the DNA size, leading to a size-based separation. On the basis of the two mechanisms, grafted polymers should have a higher efficiency as a separation medium because the comblike polymers can form many interlocking points that can prevent the chains from sliding away from each other, and thereby form a network with stabilized "pores" according to the first mechanism, or can increase the friction of the DNA−polymer complex according to the second mechanism.

Both PEO and PNIPAM can automatically coat the capillary tubing. The homo-PNIPAM and PNIPAM-*g*-PEO copolymer chains can undergo a temperature-induced volume phase transition, resulting in a viscosity-adjustable property.[19,20] A new kind of DNA separation medium, copolymers of PNIPAM-*g*-PEO with densely grafted short PEO chains ($M_w = 1000$), was synthesized, characterized, and evaluated. In this paper, the properties of high molecular weight PNIPAM-*g*-PEO copolymers with densely grafted PEO branches in 1xTBE buffer solution were characterized by NMR and laser light scattering. The DNA separation efficiency of such

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kinds of copolymers was investigated by capillary electrophoresis. The relation of DNA separation efficiency with copolymer chain conformation was discussed.

Experimental Section

Sample Preparation. *N*-Isopropylacryamide (NIPAM) (Sigma, St. Louis, MO) and 2,2-azobis(isobutyronitrile) (AIBN) (Aldrich, Milwaukee, WI) were purified by recrystallization in a benzene/hexane mixture and in ethanol, respectively. Poly(ethylene glycol) monomethyl ether monomethacrylate (M_w = 1000) was obtained from Polyscience, Inc. (Warrington, PA) and was used without further purification. A solution of NIPAM (2 g), poly(ethylene glycol) monomethyl ether monomethacrylate (0.786 g), and AIBN (10 mg) in 20 mL of benzene was placed in an ampule, and oxygen was removed by nitrogen flow. Polymerization was carried out in a sealed ampule at 50 °C for 48 h. The polymerization mixture was dissolved in 200 mL of benzene, and the polymer was precipitated by dropwise addition of ethyl ether. The polymer fractions were dried in a vacuum oven overnight. The yield of the first fraction was 1.46 g, which was used to prepare the solutions in 1xTBE buffer for laser light scattering and CE studies without further purification.

Laser Light Scattering (LLS). A standard laboratory-built laser light scattering spectrometer equipped with a BI-9000 AT digital correlator and a solid-state laser (DPSS, Coherent, 200 mW, 532 nm) was used to perform LLS studies over a scattering angular range of 20−90° and a temperature range of 22−48 °C. In static LLS, the angular dependence of the excess absolute time-averaged scattered intensity, known as the Rayleigh ratio $R_{vv}(\theta)$, was measured. For a very dilute solution, the weight-averaged molar mass (M_w) and the root-mean-square radius of gyration ($\langle R_g \rangle$) can be obtained on the basis of

$$[HC/R_{vv}(\theta)] \approx (1/M_w)[1 + {}^1/_3\langle R_g\rangle^2 q^2] \quad (1)$$

where $H = 4\pi n^2(dn/dC)^2/(N_A\lambda^4)$ and $q = (4\pi n/\lambda)\sin(\theta/2)$ with N_A, n, dn/dC, and λ being the Avogadro constant, the solvent refractive index, the specific refractive index increment, and the wavelength of light in a vacuum, respectively. In dynamic LLS, the intensity−intensity time correlation function $G^{(2)}(t)$ in the self-beating mode was measured[21]

$$G^{(2)}(t) = A[1+ \beta|g^{(1)}(t)|^2] \quad (2)$$

where A is the measured baseline, β is a coherence factor, t is the delay time, and $g^{(1)}(t)$ is the normalized first-order electric field time correlation function. $g^{(1)}(t)$ is related to the line width distribution $G(\Gamma)$ by

$$g^{(1)}(t) = \int_0^\infty G(\Gamma)e^{-\Gamma t}\, d\Gamma \quad (3)$$

By using a Laplace inversion program, CONTIN, the normalized distribution function of the characteristic line width $G(\Gamma)$ was obtained. For a pure diffusive relaxation,

$$\Gamma = D/q^2 \quad (4)$$

where D is the translational diffusive coefficient. D can be further converted into the hydrodynamic radius R_h by using the Stokes−Einstein equation:

$$D = k_B T/6\pi\eta R_h \quad (5)$$

where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the viscosity of the solvent, respectively.

Capillary Electrophoresis (CE). PNIPAM-*g*-PEO solutions were prepared by dissolving the copolymers in 1xTBE buffer (89 mM tris(hydroxymethyl)aminomethane, 89 mM boric acid, and 2 mM EDTA in deionized water) to the desired concentrations. The solution was stored at room temperature for a few days before use. The φX174/Hae III digest and pBR322/Hae III digest were purchased from New England Biolabs Inc. (Beverly, MA) and Sigma Chemical Co., respectively. All DNA digests were diluted to 10 μg/mL by using 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA).

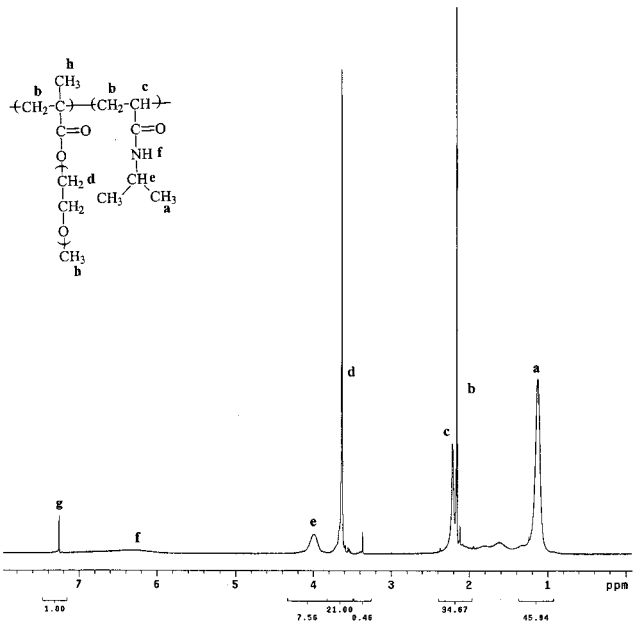

Figure 1. Typical ^{1}H NMR spectrum of PNIPAM-*g*-PEO-1 copolymer in $CDCl_3$ at 25 °C.

Separation was performed using the lab-made capillary electrophoresis system with laser-induced fluorescence detection. The detailed description can be found elsewhere.[22] A certain length fused silica capillary (Polymicro Technologies; Phoenix, AZ) with i.d./o.d. = 100 μm/364 μm was flushed with 1 N HCl for 10 min. A detection window of 1.5 mm width was opened at a required length from the cathodic end by stripping the polyimide coating off the capillary with a razor blade. Both cathode and anode reservoirs (1.6 mL volume) were filled with 1xTBE buffer and 3 μg/mL ethidium bromide (Sigma Chemical Co.). The copolymer solution was injected into the capillary tubing by using a 50 μL syringe. The capillary tubing was then assembled onto the holder, and a prerun at a constant electric field strength of 200 V/cm was used to introduce the fluorescent dye into the separation medium and to stabilize the current. The DNA sample was electrokinetically injected into the capillary. The voltage was generated by using a PCI-MIO-16E-4 multifunction I/O board (National Instrument, Austin, TX) together with a Trek PM0610 amplifier. The fluorescence was detected by Hamamatsu R928 photomultiplier tube (Rockway, NJ), and data were acquired by the same I/O board and processed by a Pentium personal computer.

Results and Discussion

The amount of PEO in the PNIPAM-*g*-PEO copolymers can be estimated from ^{1}H NMR spectroscopy. Figure 1 shows a typical ^{1}H NMR spectrum of the PNIPAM-*g*-PEO-1 copolymer in $CDCl_3$ recorded by a Varian Gemini 2000 (300 MHz) spectrometer. The resonances of two methyl groups (h) on poly(ethylene glycol) monomethyl ether monomethacrylate were ignored here because the number of grafted PEO chains was much less than that of NIPAM repeating units. The protons (b, c) on the copolymer backbone have a chemical shift of 2.0−2.2 ppm. The methylene protons (d) of PEO have a chemical shift of 3.63 ppm. The peaks at 1.1 and 4.0 ppm are assigned to the methyl proton (a) and methylene protons (e) in the *N*-isopropyl group, respectively. The peak above 7 belongs to the solvent resonance, and the proton connected to nitrogen has a

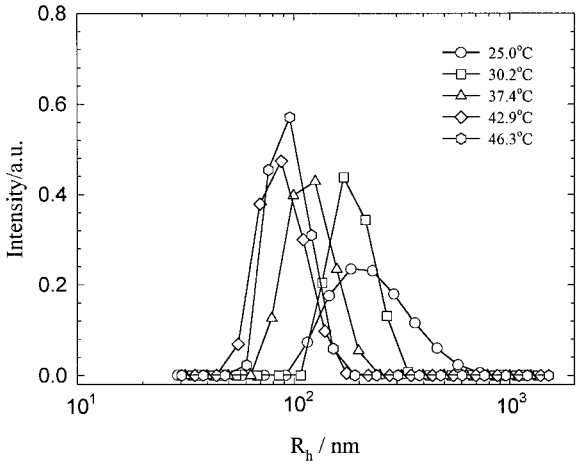

Figure 2. Temperature dependence of hydrodynamic radius distribution of PNIPAM-*g*-PEO-1 copolymer solution in 1xTBE buffer at $\theta = 20°$ and $C = 1.0 \times 10^{-6}$ g/mL.

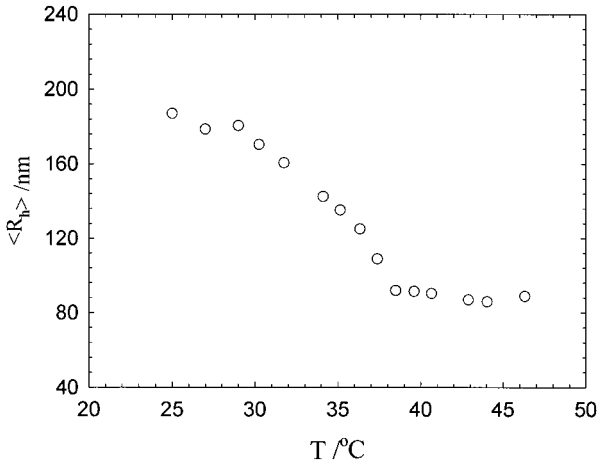

Figure 3. Temperature dependence of average hydrodynamic radius $\langle R_h \rangle$ of PNIPAM-*g*-PEO-1 copolymer in 1xTBE buffer solution at $\theta = 20°$and $C = 1.0 \times 10^{-6}$ g/mL.

very broad peak at about 6.1. From the integral areas, both the ratio of EO/NIPAM and the number density of the PEO chains on the backbone can be estimated. By using the peak area ratio of peaks located at 3.63 and 4.0, or 3.63 and 1.1 ppm, a molar ratio of EO/NIPAM of 0.7 was obtained, which corresponded to a total PEO content of about 21 wt % in the PNIPAM-*g*-PEO-1 copolymer, where the last digit denotes sample 1. The number of the repeating units in the grafted PEO (M_w = 1000) was 22. Thus, there was, on average, one grafted PEO chain per 30 repeating units in the PNIPAM backbone chains for the PNIPAM-*g*-PEO-1 copolymer. Similarly, we got the compositions of PNIPAM-*g*-PEO-2 copolymer with a total PEO content of 5 wt % and one grafted PEO chain per 150 repeating units on the backbone chain.

Figure 2 shows the temperature dependence of the hydrodynamic radius (R_h) distribution of the PNIPAM-*g*-PEO-1 copolymer in 1xTBE buffer at $C = 1 \times 10^{-6}$ g/mL and $\theta = 20°$. With the increase in temperature, the distribution became narrower and shifted to the small R_h value. These results indicated a temperature-induced "coil-to-globule" transition occurring for the PNIPAM-*g*-PEO copolymers in 1xTBE buffer. At low temperatures, the copolymers existed as an expanded coil conformation. With increasing temperature, the PNIPAM backbone chains started to shrink gradually until reaching the collapsing limit, where the conformation of the copolymer chains should be similar to the globule state, as will be evidenced later. Therefore, at high temperatures (T > 37.4 °C), the R_h distribution became narrower.

Figure 3 shows a plot of the average hydrodynamic radius $\langle R_h \rangle$ versus temperature for the PNIPAM-*g*-PEO-1 in 1xTBE buffer at $C = 1 \times 10^{-6}$ g/mL and $\theta = 20°$. Three stages were observed: (1) at T < 30.5 °C, $\langle R_h \rangle$ decreased slightly with increasing temperature, indicating that the solvent quality became poorer and the polymer chains shrank gradually with increasing temperature; (2) in the temperature range of 31.8−38.5 °C, $\langle R_h \rangle$ decreased fast with increasing temperature, indicating that the copolymer chains underwent a "coil-to-globule" transition; (3) at T > 38.5 °C, $\langle R_h \rangle$ remained nearly unchanged with further increase in temperature. In comparison with dilute aqueous solutions of high molecular weight ($M_w \sim 10^7$ g/mol) homo-PNIPAM or PNIPAM-*g*-PEO with a relatively low number density of grafted PEO chains on the backbone chain,[19,20] the first and third stages were quite similar, while the second stage of the "coil-to-globule" transition occurred in a much broader temperature range; namely, the homo-PNIPAM or PNIPAM-*g*-PEO with a low number density of PEO branches showed a sharp transition within 1−2 °C. At low temperature, both PNIPAM and PEO are hydrophilic so that the whole copolymer chain has an expanded random coil conformation. An increase of temperature made the 1xTBE solvent quality poorer for PNIPAM backbone chains. Thus, $\langle R_h \rangle$ became smaller. When the temperature was increased to the low critical solution temperature (LCST) of PNIPAM, the PNIPAM chains would undergo a sharp collapse from coil to globule conformation within 1−2 °C. However, the PEO chains would still be hydrophilic. Therefore, the extent of collapse and the transition temperature range of PNIPAM-*g*-PEO copolymer should depend on the PEO content and the number density of grafted PEO chains. The interlock points between grafted PEO chains, and the copolymer backbone chains made the chains relatively stiff. The more the grafted PEO chains, the stiffer the copolymer backbone chains and thus the more difficult for the copolymer chains to change conformation. Furthermore, with the gradual shrink of the copolymer chains, the grafted hydrophilic PEO chains prefer to locate on the surface of the particle, which also prevents the copolymer chains from collapse. To induce the collapse of the copolymer chains, the temperature has to be raised more. Eventually, the number density of the hydrophilic PEO chains per unit area on the surface of the contracted particle became so high that the PNIPAM chains located inside the particle would not collapse anymore; thus, the $\langle R_h \rangle$ kept nearly unchanged in the third stage. As shown by the NMR results in Table 1, there is one grafted PEO chain per 30 and 150 repeating units of the backbone chain in the PNIPAM-*g*-PEO-1 and PNIPAM-*g*-PEO-2 copolymer chains, respectively, while for the PNIPAM-*g*-PEO copolymers in ref 20 with a sharp collapse transition of 1−2 °C, there is one PEO chain per about 800 repeating units of the backbone chain. It means that the number density of the grafted PEO chains in our PNIPAM-*g*-PEO copolymers is much higher than that of the sample in ref 20. That is why the temperature range of the "coil-to-globule" transition in our system was much broader, and the total volume change of the copolymer chains

Table 1. NMR and LLS Characterization Results of the PNIPAM-*g*-PEO Copolymers

sample	M_w/(g/mol)	PEO content (wt %)	$n_{r.u.}$ per PEO[a]	$\langle R_g\rangle/\langle R_h\rangle_0$ (25 °C)	$\langle R_g\rangle/\langle R_h\rangle_0$ (42 °C)
PNIPAM-*g*-PEO-1	6.4×10^7	21	30	1.4	1.1
PNIPAM-*g*-PEO-2	1.1×10^7	5.0	150	1.5	1.0

[a] $n_{r.u.}$ per PEO represents the number of repeating units on backbone chain with one PEO chain.

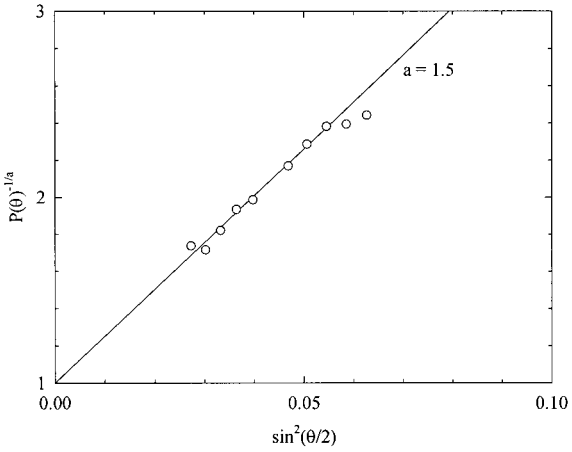

Figure 4. Plot of $P(\theta)^{-1/a}$ versus $\sin^2(\theta/2)$ with $a = 1.5$ for PNIPAM-*g*-PEO-1 copolymer in 1xTBE buffer at $T = 25$ °C and $C = 1.0 \times 10^{-6}$ g/mL, where $P(\theta)$ is the scattering function.

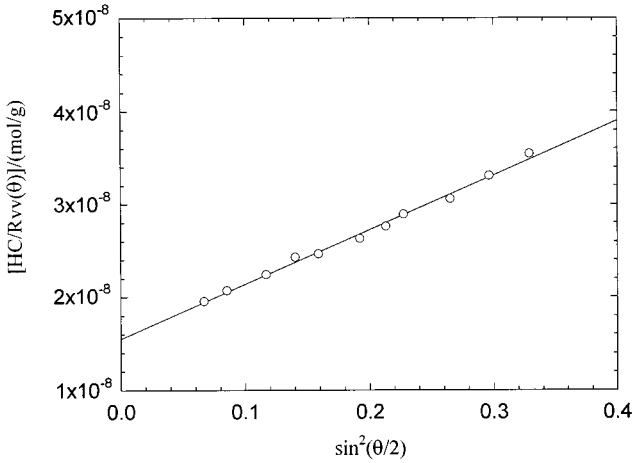

Figure 5. Angular dependence of excess scattered intensity $[HC/R_{vv}(\theta)]$ of PNIPAM-*g*-PEO-1 copolymer in 1xTBE buffer at $T = 42$ °C and $C = 1.0 \times 10^{-6}$ g/mL.

induced by the conformation transition was relatively smaller.

To evaluate the densely grafted PNIPAM-*g*-PEO copolymer chain conformation, a ratio of radius of gyration to hydrodynamic radius should be determined. The extrapolation of the q^2 dependence of the apparent $\langle R_h\rangle$ of the PNIPAM-*g*-PEO-1 copolymer chains in 1xTBE buffer solution at a scattering angular range of 20−30° gave us a $\langle R_h\rangle_0$ value of 244 nm at $T = 25$ °C and $C = 1 \times 10^{-6}$ g/mL. On the basis of a random coil conformation for the PNIPAM-*g*-PEO in water with $R_g/R_h \sim 1.5$ at 25 °C,[20] the R_g value of the copolymer chains could be estimated to be about 360 nm at the same temperature. The use of a low scattering angular range of $\theta < 10°$ was necessary for the static light scattering measurements in order to get an accurate determination on M_w and R_g at $qR_g < 1$. However, our present instrument could not reach such a low scattering angular range. Nevertheless, we could adopt an approximate analysis method for static LLS data to estimate the R_g value of large molecular weight polymers.[23] The analysis is based on the Debye scattering function $P(X)$ for narrowly distributed random flight polymer chains, where $X = \langle R_g\rangle^2 q^2 P(X)$ can be numerically approximated by the single term $(1 + X/3a)^{-a}$ at the exponent $a = 1.5$ when the X value is relatively small, e.g., $X < 8$. Figure 4 shows a plot of $P(\theta)^{-1/a}$ versus $\sin^2(\theta/2)$ for the PNIPAM-*g*-PEO-1 copolymer with $a = 1.5$. The curve was obtained within a scattering angular range of 19−29°. From the initial slope ($19° \le \theta \le 27°$), an $\langle R_g\rangle$ value of 338 nm was obtained. With this $\langle R_g\rangle$ value, an X value of 6.2 was obtained at $\theta = 27°$, indicating that the scattering angular range of 19−27° corresponded to a range of X values that were relatively small. An estimated $\langle R_g\rangle$ value of 3.4×10^2 nm could give us a ratio of $\langle R_g\rangle/\langle R_h\rangle_0 = 1.4$. This ratio is relatively small for a random coil polymer chain. However, considering the polydispersity of the PNIPAM-*g*-PEO-1 copolymer, the uncertainty of the estimated $\langle R_g\rangle$ value should be acceptable.

We could also measure the M_w of the PNIPAM-*g*-PEO-1 copolymer after the copolymer chains underwent the "coil-to-globule" transition, where the $\langle R_g\rangle$ value of the copolymer chains would be much smaller and the expansion of the Debye function would remain valid at smaller X values. Figure 5 shows the angular dependence of the excess scattered intensity of $HC/R_{vv}(\theta)$ for the PNIPAM-*g*-PEO-1 copolymer in 1xTBE buffer at $C = 1 \times 10^{-6}$ g/mL and $T = 42$ °C, under which the copolymer chains reached a collapsed limit. At such a dilute concentration, a M_w of 6.4×10^7 g/mol and a $\langle R_g\rangle$ of 107 nm could be determined from the intercept and the slope of the plot at $T = 42$ °C. By combining $\langle R_g\rangle$ with the $\langle R_h\rangle_0$ value of 97 nm of the PNIPAM-*g*-PEO-1 chains in 1xTBE buffer at $T = 42$ °C, a ratio of $\langle R_g\rangle/\langle R_h\rangle_0 = 1.1$ was obtained. This ratio is larger than the value of 0.77 for a fully collapsed chain globule with a uniform density. The PNIPAM-*g*-PEO-2 copolymer chains were relatively short; all the M_w, $\langle R_g\rangle$, and $\langle R_h\rangle$ values of the PNIPAM-*g*-PEO-2 copolymer in both expanded and collapsed states could be measured by a combination of static and dynamic LLS. The results are also summarized in Table 1. The $\langle R_g\rangle/\langle R_h\rangle_0$ ratio of 1.5 for the PNIPAM-*g*-PEO-2 chains at 25 °C indicated a random coil conformation in 1xTBE buffer solution, while the $\langle R_g\rangle/\langle R_h\rangle_0$ value of 1.0 for the copolymer at $T = 42$ °C is still larger than the value of 0.77, indicating that the densely grafted PNIPAM-*g*-PEO chains did not collapse to a globular state with a uniform density. The higher number density of the grafted PEO chains in our copolymers not only made the backbone chain stiff but also provided the gradually contracted chains with a more complete hydrophilic PEO shell that could limit the copolymer chains from collapsing further. Consequently, the $\langle R_g\rangle/\langle R_h\rangle_0$ values in the collapsing limit of our PNIPAM-*g*-PEO copolymers were relatively high. However, the PNIPAM-*g*-PEO copolymer with a low number density of grafted PEO chains could collapse to a globule state with $\langle R_g\rangle/\langle R_h\rangle \sim 0.74$.[20]

After the NMR and LLS characterizations, we have a clearer picture about the properties of the two PNIPAM-*g*-PEO copolymers in 1xTBE buffer. At low temperatures ($T < 30$ °C), the copolymer chains had a

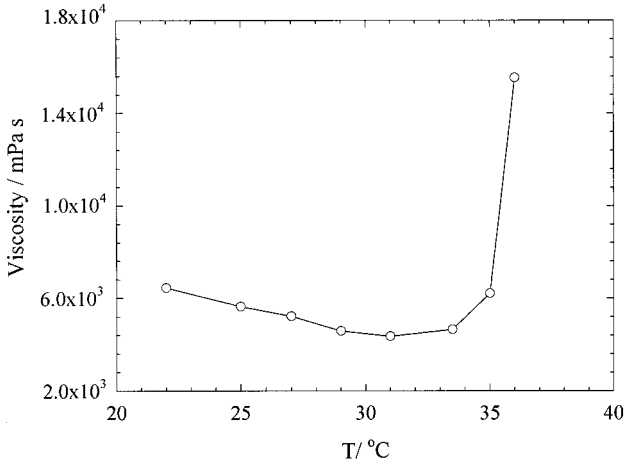

Figure 6. Temperature dependence of viscosity of PNIPAM-*g*-PEO-1 copolymer in 1xTBE buffer solution with $C = 10$ wt %.

random coil conformation with densely grafted PEO branches. With a further increase in temperature, the copolymer chains would shrink and collapse to a globule state ($\langle R_g \rangle / \langle R_h \rangle_0 \sim 1.0$) with PNIPAM staying inside the core and the hydrophilic PEO branches on the surface. Such a special chain conformation change, as will be shown in the following, could make the copolymers have several advantages as a viscosity adjustable DNA separation medium that could coat the capillary channel automatically and had a high resolution although the collapse of the copolymer chains could eventually destroy the high sieving ability for DNA separation.

The DNA separation was carried out in polymer solutions at high concentration (e.g., 10 wt %); the viscosity of the 10 wt % PNIPAM-*g*-PEO-1 solution in 1xTBE buffer was measured by using a Brookfield (Stouhgton, MA) cone/plate viscometer over a temperature range of 22−36 °C. The temperature dependence of the viscosity in Figure 6 showed three stages: (1) at $T < 31$ °C, the viscosity decreased slowly with increasing temperature; (2) at $T > 31$ °C, the viscosity increased slowly; (3) at $T > 35$ °C, the viscosity increased dramatically with a sharp jump. These results can be understood as follows. At low temperature, both PNIPAM backbone and PEO chains are hydrophilic. With a high concentration of 10 wt %, the long PNIPAM-*g*-PEO chains could overlap and form a network with grafted PEO chains, forming combs and suspending inside the mesh of the network. When increasing the temperature, the PNIPAM backbone chains contracted gradually so that the hydrodynamic interactions between copolymer chains and solvent were weakened and the viscosity decreased. At $T > 31$ °C, the collapse of the copolymer chains became much faster with increasing temperature, and some interchain aggregation would be formed. With a further increase in temperature ($T > 35$ °C), the PNIPAM backbone chains became more hydrophobic so that the interchain aggregation became very strong at high concentrations (e.g., 10 wt %). Thus, the viscosity increased dramatically.

Figure 7 shows a comparison of the electropherogram of φX174/Hae III digest by using 10 wt % PNIPAM, PNIPAM-*g*-PEO-2, and PNIPAM-*g*-PEO-1 as a separation medium at $T = 25$ °C. Obviously, homo-PNIPAM is not a good DNA separation medium, where all the 11 peaks showed bad shape with 271 and 281 bp combined together and low signal-to-noise ratio. In

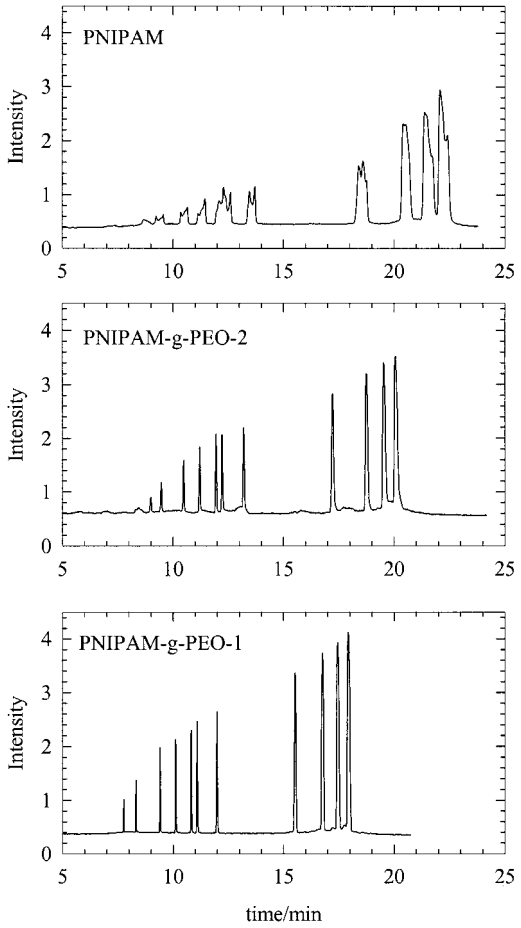

Figure 7. Electropherograms of φX174/Hae III digest by using 10 wt % PNIPAM, PNIPAM-*g*-PEO-2, and PNIPAM-*g*-PEO-1 in 1xTBE buffer solutions at 25 °C as separation medium, respectively, under the following conditions: 100 μm i.d. capillary, effective length = 10 cm, total length = 12 cm, electric field strength = 200 V/cm, electrokinetic injection at 300 V/cm for 10 s. Peak identifications from left to right in bp are 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1353.

contrast, when a small amount of PEO chains (5 wt %, an average of one PEO chain per 150 repeating units of backbone chain) were grafted to the PNIPAM backbone chains, the PNIPAM-*g*-PEO-2 matrix as a DNA separation medium was dramatically improved. All the fragments, ranging from 72 to 1353, were clearly separated within 21 min, although the resolution for large DNA fragments was not very good and the baseline had a slight fluctuation. When the number density of grafted PEO chains was raised to an average of one PEO chain per 30 repeating units (21 wt %) of the PNIPAM-*g*-PEO-1 backbone chain, the efficiency of the DNA separation was further enhanced. Very high resolution, short separation time (18 min), high signal-to-noise ratio, and flat baseline were achieved. The comparison clearly showed that the grafted short PEO chains on the copolymer chains could play an important role in DNA separation.

To confirm the high sieving ability of PNIPAM-*g*-PEO-1 copolymer as a DNA separation medium, another kind of DNA sample, pBR322/Hae III digest containing 22 fragments ranging from 8 to 587 bp including two fragments (123 and 124 bp) with 1 bp difference, was analyzed by using CE. Figure 8 shows the electropherogram of PBR322/Hae III digest sample separated in the 10 wt % PNIPAM-*g*-PEO-1/1xTBE buffer solution at *T*

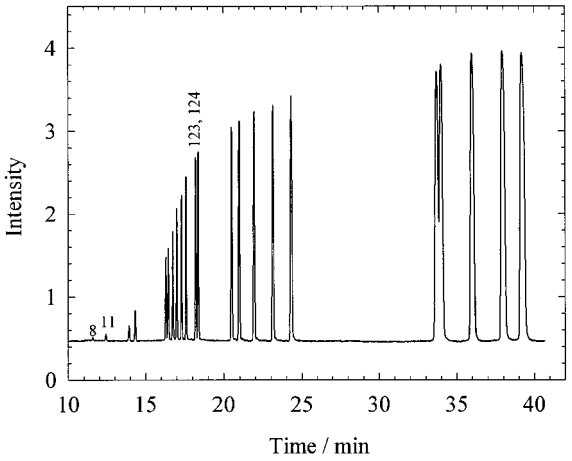

Figure 8. Electropherogram of pBR322/Hae III digest by using 10 wt % PNIPAM-*g*-PEO-1 in 1xTBE buffer solution as a separation medium at room temperature. All the other conditions are the same as in Figure 7. Peak identifications from left to right in bp are 8, 11, 18, 21, 51, 57, 64, 80, 89, 104, 123, 124, 184, 192, 213, 234, 267, 434, 458, 504, 540, and 587.

= 25 °C. To detect the small fragments such as 8 and 11 bp, a large amount of DNA sample was injected into the capillary tubing. It can be seen that the fragments show good resolution; especially, a baseline separation for the doublet fragments of 123/124 bp peaks was achieved. In comparison with the best separation results reported so far for the same DNA sample by using linear PAM as a separation medium,[4] the separation medium of PNIPAM-*g*-PEO-1 copolymers has much less migration time and much stronger signal-to-noise ratio. For example, all the DNA fragments were detected in Figure 7, while the first two fragments (8 and 11 bp) were missed when using a linear PAM (or PEO mixture) as a separation medium.[4] The separation results from the PNIPAM-*g*-PEO-1 matrix were also better than those obtained from the separation medium of $PEO_{99}PPO_{69}$-PEO_{99} copolymers.[14]

The dramatic enhancement of the DNA separation efficiency by using the PNIPAM-*g*-PEO copolymers as a separation medium could be ascribed to the special chain conformation of the copolymers. As discussed above, both PNIPAM and PEO are hydrophilic at low temperatures, and the long PNIPAM-*g*-PEO copolymer chains could form a network by chain overlap at high concentrations. The formed network was relatively stable because the densely grafted PEO chains made the backbone chains relatively stiff, and the interactions of the grafted PEO chains prevented the overlapped polymer chains from sliding past each other. Thus, the mesh size was relatively more stable, which could increase the sieving ability. Furthermore, the entanglement of DNA with the hydrophilic copolymer chains should also be stronger since the entanglement times for copolymer chains should be longer. The strengthened interactions of the DNA−polymer complex could also lead to higher resolutions.

Although the PNIPAM-*g*-PEO copolymers demonstrated an excellent performance as a DNA separation medium at low temperatures (i.e., $T \leq 25$ °C), an increase in temperature would destroy the good sieving ability of the medium. Figure 9 shows the temperature effects on the separation efficiency for φX174/Hae III digest sample in 10 wt % PNIPAM-*g*-PEO-1/1xTBE buffer solution. By increasing the temperature from 25

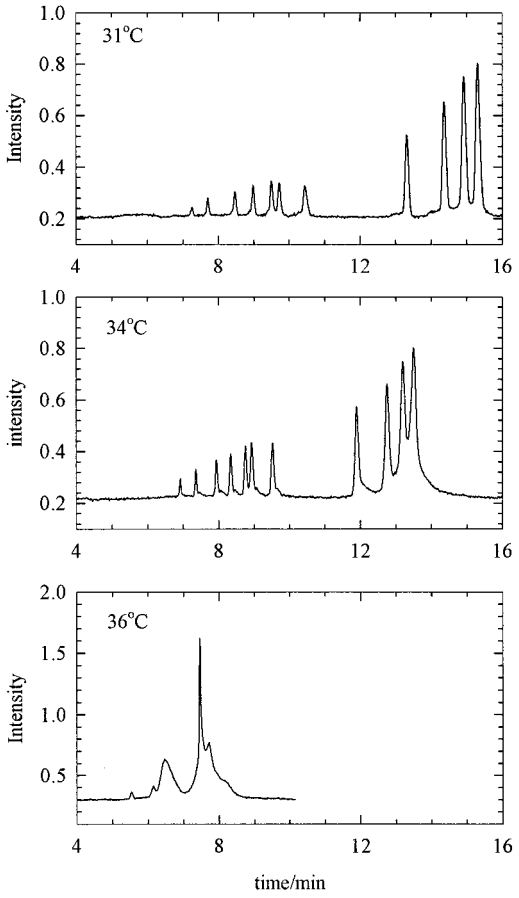

Figure 9. Electropherograms of φX174/Hae III digest by using 10 wt % PNIPAM-*g*-PEO-1 in 1xTBE buffer solution as a separation medium at different temperatures. All the other conditions are the same as in Figure 7.

°C (Figure 7c) to 36 °C during which the copolymer chains underwent a broad "coil-to-globule" transition, the resolution underwent a dramatic change. At 25 °C, a baseline separation of the 11 fragments of φX174/Hae III digest was achieved within 18 min, and the peaks were narrow and sharp (Figure 7c). At 31 °C, the 11 fragments were observed with broad bands and low intensity. At 34 °C, the resolution became even poorer, especially for the large fragments. By increasing the temperature further to 36 °C, the resolution became so bad that the fragments could not be identified except for the first peak. In addition, with the resolution and peak shape becoming bad, the migration time became fast; i.e., the time decreased from 18 min at 25 °C to 8.5 min at 36 °C. The negative effects of temperature on the DNA separation medium of PNIPAM-*g*-PEO copolymers could also be explained in terms of the conformation change of the copolymers. With increasing temperature, the PNIPAM backbone chains became relatively more hydrophobic and shrank gradually. When the DNA fragments are migrated through the copolymer network under an applied electric field, the DNA chains prefer to entangle with the PEO chains since DNA is hydrophilic. On a further increase in temperature, the PNIPAM chains collapsed even more. The collapsed copolymer chains would aggregate together to form a core with hydrophobic PNIPAM staying inside and hydrophilic PEO on the shell. The hydrophobic core does not have any sieving ability for DNA since DNA is hydrophilic and will not go through it, while the PEO corona is the only path for DNA. Because

the PEO chains were very short (22 repeating units) and could not form a network, it was very difficult for them to entangle with DNA strongly. Thus, the separation medium of aggregated particles formed by the collapsed PNIPAM-*g*-PEO chains showed poor resolution and fast migration time.

Conclusions

High-M_w PNIPAM-*g*-PEO copolymers ($M_w > 1 \times 10^7$ g/mol) with very densely grafted PEO chains (one PEO chain per 30 repeating units of backbone chain) could undergo a very broad temperature-induced "coil-to-globule" transition. At low temperatures, the long copolymer chains existed in a random coil conformation with densely grafted PEO branches in 1xTBE buffer solution. At high concentrations (i.e., ≥10 wt %), these expanded copolymer chains could form a network with relatively stable pores by chain overlap, yielding a high sieving ability with 1 bp resolution when used for dsDNA separation in capillary electrophoresis. The densely grafted PEO chains played an important role in the high sieving ability for DNA separation. When the temperature was increased above the LCST of the copolymers, the copolymer chains collapsed to nanoparticles with PNIPAM inside the core and the hydrophilic PEO chains on the shell. Such a conformation made the copolymers lose the good sieving ability as a DNA separation medium.

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