Thermogelling Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) Disulfide Multiblock Copolymer as a Thiol-Sensitive Degradable Polymer

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We report a reverse thermogelling poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer as a thiol-sensitive biodegradable polymer. The poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) aqueous solutions studied in this research underwent sol−gel−sol or sol−gel−sol−gel transition depending on the molecular weight and concentration of the polymer, whereas the corresponding disulfide multiblock copolymer aqueous solutions underwent sol−gel transition as the temperature increased in a range of 0−60 °C. The hydrophobic dye solubilization and dynamic light scattering of the polymer aqueous solution suggest that the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s undergo unimer (3 nm) to micelle (12 nm) transition, whereas the disulfide multiblock copolymers undergo unimer (6 nm) to aggregated polymer (600 nm) transition as the temperature increases. The gel duration increased from 6 h (poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)) to more than 12 days (the corresponding disulfide multiblock copolymer) in phosphate buffer saline, and the gel duration of the latter depended on the glutathione concentration of the medium. The model drug, paclitaxel, was released from the in-situ-formed poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer gel in a glutathione concentration-sensitive manner.

Introduction

Reverse thermogelling polymers enable pharmaceutical agents or cells to be easily entrapped and form a depot by a simple syringe injection at a target site, where the depot acts as a sustained drug delivery system or a cell-growing matrix for cells or stem cells.[1−4] Poly(ethylene oxide)/poly(propylene oxide) triblock and multiblock copolymers, poly(ethylene oxide)/poly(butylene oxide) di- and triblock copolymers, poly(ethylene glycol)/poly(lactic acid-*co*-glycolic acid) triblock and graft copolymers, poly(ethylene glycol)/poly(propylene fumarate), chitosan/glycerol phosphate, polyphosphazene, and poly(ethylene glycol)/polycaprolactone have been reported as thermogelling polymer aqueous systems.[5−13] They are a low viscous sol at room temperature or lower and form a gel at body temperature (37 °C). In particular, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers have been extensively investigated on their unique thermogelling phase behavior as well as drug delivery applications.[14,15] However, their short gel duration and nonbiodegradability limit applications of the material.

Disulfide bonds are found in many proteins and play an important role in keeping the structural stability and rigidity of the native proteins.[16] They can reversibly undergo reduction to thiols depending on the environmental thiol concentration. Depending on the location in our body as well as pathological condition, the thiol concentration varied. The thiol concentration is about 10 mM in the cytosol, whereas it is about 10 μM in the plasma.[17,18] The thiol concentration increases by as much as 7 times around some tumors.[19] On the basis of this fact, the disulfide bond has been introduced to polymeric systems such as the degradable poly(ethylene glycol) nanoparticle, the poly(ethylene glycol)-drug conjugate as a degradable PEGylated drug, the DNA detachable poly(L-lysine) for DNA delivery, the poly(ethylene glycol) detachable phospholipids as thiol-sensitive destabilizable phospholipids, the magnetic resonance imaging agent conjugate for tumor imaging, and the in-situ cross-linkable polymer for cell encapsulation.[18,20−25]

We report a poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer as a new reverse thermogelling polymer that is expected to prolong the gel duration of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) itself and undergo thiol-dependent degradation and drug release. The drug is expected to be released from the long durable gel, and the polymer can be internalized into the cell and degraded therein, followed by being eliminated by the kidney. In this paper, the phase behavior and micellization properties of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) and the corresponding disulfide multiblock copolymer were compared. In addition, the degradation and model drug release from the in-situ-formed disulfide multiblock copolymer hydrogel were investigated as a function of thiol concentration in the release medium.

Materials and Experimental Methods

Materials. Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s with the same PEO weight percent (P65, P85, and P105) were purchased from BASF and used as received. *p*-Toluenesulfonyl chloride, *N*,*N*-dimethylaminopyridine, triethylamine, *N*,*N*-dimethylformamide, 1,6-diphenyl-1,3,5-hexatriene, potassium thioacetate, Tween 80, and ammonia (2.0 M in methanol) were used as received from Aldrich.

Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide). *Disulfide Multiblock Copolymer Synthesis*. The multiblock copolymer was prepared by oxidative coupling of the corresponding dithio-poly-

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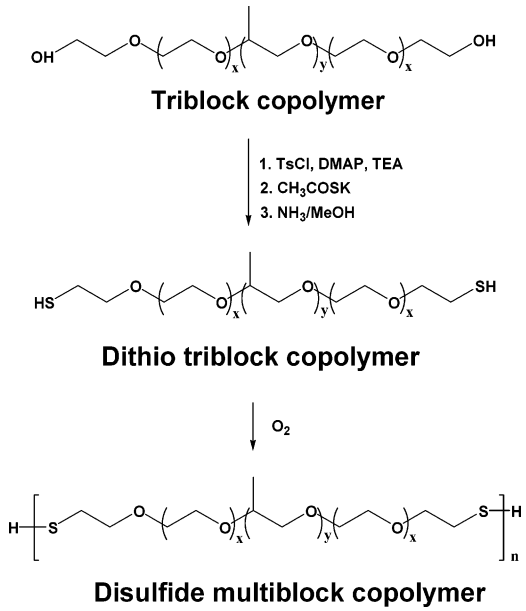

Figure 1. Synthetic scheme of a poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer.

Table 1. List of Polymers Studied

polymer[a]	MW[b]	PPO MW[b]	composition[b]	M_n[c]	M_w/M_n[c]
P65	3400	1700	$(EO)_{19.3}(PO)_{29.3}(EO)_{19.3}$	5400	1.1
P85	4600	2300	$(EO)_{26.1}(PO)_{39.7}(EO)_{26.1}$	6500	1.2
P105	6500	3250	$(EO)_{36.9}(PO)_{56.0}(EO)_{36.9}$	8900	1.2
P65-SS				22 400	1.5
P85-SS				28 400	1.4
P105-SS				31 700	1.4

[a] P65-SS, P85-SS, and P105-SS are the disulfide multiblock copolymers prepared from P65, P85, and P105, respectively. [b] As provided by BASF.[30] The weight ratio of the poly(ethylene oxide) (PEO) block to the poly(propylene oxide) (PPO) block is 1.0 for all poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s used in this study. [c] Determined by gel permeation chromatography using poly(styrene) standards.

(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (Figure 1).[18,26] For example, the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer (P85-SS in Table 1) was synthesized by the following procedure. First, the P85 (9.2 g, 2.0 mmol) was dissolved in anhydrous toluene (50 mL), and the solvent was distilled off to a final volume of 10 mL to remove the residual water adsorbed to the polymer. After cooling the reaction mixture, methylene chloride (4.0 mL), 4-(dimethylamino)pyridine (0.122 g, 1.0 mmol), *p*-toluenesulfonyl chloride (1.1439 g, 6.0 mmol), and triethylamine (1.0 mL, 7.2 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. After adding chloroform (100.0 mL), the product was washed three times with water (3 × 20 mL; pH 5.0) saturated with sodium chloride. The organic layer was dried with magnesium sulfate (anhydrous), and solvent was evaporated to separate the tosylated poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide). The yield was 75%.

^{1}H NMR ($CDCl_3$) of tosylated poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide): δ 1.35 ($-OCH_2CH(\boldsymbol{CH_3})-$), 2.40 ($\boldsymbol{CH_3}-C_6H_4-SO_3-$), 3.10−3.80 ($-O\boldsymbol{CH_2}CHCH_3-$) and ($-O\boldsymbol{CH_2}CH_2-$), 4.10 ($-OCH_2\boldsymbol{CH_2}-O_3SC_6H_4-CH_3$), 7.30 and 7.80 ($CH_3-\boldsymbol{C_6H_4}-SO_3-$).

Second, *N*,*N*-dimethylformamide (25.0 mL) was added to dissolve the tosylated poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (7.4 g, 1.6 mmol), and potassium thioacetate (0.685 g, 6.0 mmol) was added. The reaction mixture was stirred at room temperature for 7 h. The *N*,*N*-dimethyl formamide was removed under vacuum. Water (50.0 mL) was added, and the product was extracted three times by chloroform (3 × 100 mL). The solvent was evaporated, and the product was separated to prepare the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) dithioacetate. The yield was 73%.

^{1}H NMR ($CDCl_3$) of poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) dithioacetate: δ 1.35 ($-OCH_2CH(\boldsymbol{CH_3})-$), 2.20 ($-SCO\boldsymbol{CH_3}$), 3.05 ($-OCH_2\boldsymbol{CH_2}-SCOCH_3$), 3.10−3.80 ($-O\boldsymbol{CH_2}-CHCH_3-$ and $-O\boldsymbol{CH_2}CH_2-$).

Third, ammonia/methanol solution (2.0 M, 10 mL) was added to the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) dithioacetate (7.3 g, 1.59 mmol) and stirred at room temperature for 12 h. Oxygen was bubbled through the solution for 10 min, and the reaction mixture was stirred under an oxygen atmosphere at room temperature for 3 days. The solvent was evaporated, the reaction mixture was dissolved in methylene chloride, and the product was precipitated into diethyl ether/*n*-hexane (1/5 v/v). The residual solvent was removed under vacuum. The yield was 87%.

^{1}H NMR ($CDCl_3$) of poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer: δ 1.35 ($-OCH_2-CH\boldsymbol{CH_3}-$), 2.85 ($OCH_2\boldsymbol{CH_2}-SS-$), 3.1−3.8 ($-O\boldsymbol{CH_2}CHCH_3-$ and $-O\boldsymbol{CH_2}CH_2-$).

Other poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymers (P65-SS and P105-SS in Table 1) were prepared in a similar manner from the corresponding polymers of P65 and P105, respectively.

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 distributions of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s and their disulfide multiblock copolymers. Tetrahydrofuran was used as an eluting solvent. Polystyrenes in a molecular weight range of 800−50 000 Daltons were used as molecular weight standards. Styragel HMW 6E and HR 4E columns (Waters) were used in series.

NMR Study. A 250 MHz NMR spectrometer (9503DPX; Bruker) was used for ^{1}H NMR (in $CDCl_3$) to study the composition of the polymers, and a 500 MHz NMR spectrometer (Unity-inova 500 MHz; Varian) was used for ^{13}C NMR to see spectral changes of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) and its disulfide multiblock copolymer (30 wt % in D_2O) as a function of temperature.

Test-Tube Inverting Method. The sol−gel transition temperature was determined by the test-tube inverting method.[27,28] The 4 mL vials (diameter 1.1 cm) containing 0.5 mL of a polymer aqueous suspension were dissolved in a 4 °C bath overnight. The transition temperatures were determined by a flow (sol)−no flow (gel) criterion when the vial was inverted with a temperature increment of 1 °C per step. The transition temperature in the phase diagram is an average of three measurements per each point.

Falling Ball Method. The sol−gel transition of the polymer aqueous solution was also investigated by the falling ball method.[29,30] The aqueous polymer solution (30 wt % except for P65 (40 wt %)) and steel ball (diameter (D) = 1.6 mm, density (ρ_s) = 6.4 g/cm^3) were put in the NMR tube with a diameter of 4.2 mm. The time for the steel ball to fall 4.0 cm was measured with a temperature increment of 1 °C per step. The dynamic viscosity (μ) of the polymer solution can be calculated by the following equation

$$\mu = (\gamma_s - \gamma_f)D^2/(18\upsilon)$$

The velocity of the falling ball (υ) was calculated by the transit distance (4.0 cm) divided by the transit time. The density of the fluid (ρ_f) was assumed to be 1.0 g/cm^3. The specific weight of the sphere (γ_s) = $\rho_s g$ and the fluid (γ_f) = $\rho_f g$ were calculated from the gravitational acceleration (g = 980 cm/s^2) and density of the sphere and fluid. At sol−gel transition temperature, the dynamic viscosity of the fluid abruptly increases.

Dye Solubilization. 1,6-Diphenyl-1,3,5-hexatriene (DPH) solution in methanol (10 μL at 0.4 mM) was injected into an aqueous polymer solution (1.0 mL) at a polymer concentration of 0.5 wt %. The absorption spectra of these solutions were recorded from 320 to 400 nm to see the solubilization of the dye. The increase in the absorbance

at 340, 355, and 378 nm is a typical phenomenon of the solubilization of the dye.[31−32]

Dynamic Light Scattering. The apparent sizes of the polymers (P85 and P85-SS) were studied by a dynamic light scattering instrument (Zetasizer nano ZS; Malvern) as a function of temperature at a concentration of 0.5 wt % in water. A He−Ne laser operating at 633 nm was used as a light source. Measurements of 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. The decay rate distributions were transformed to an apparent diffusion coefficient. From the apparent diffusion coefficient, the apparent hydrodynamic radius of the polymer or polymer aggregates was obtained by the Stokes−Einstein equation.

Duration of the In-Situ-Formed Gel. Aqueous solutions (30 wt %) of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P85) and the disulfide multiblock copolymer (P85-SS) were prepared at 4 °C. The polymer aqueous solution (0.5 mL) was injected through a 21-gauge syringe into a 4.0 mL vial (inner diameter = 11 mm), which was thermostated at 37 °C to form a gel. After 2 min, 3.0 mL of the phosphate buffer saline (150 mM, pH 7.4) at 37 °C was added to the preformed gel. The concentration of the glutathione in the phosphate buffer saline was varied from 0, 10 μM, 10 mM, and 50 mM. The vial was shaken at 90 strokes/min. The medium was replaced by a fresh one (3.0 mL) at designated sampling intervals, and the height of the gel was measured.

Degradation of the Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) Disulfide. The same procedure was followed to form a P85-SS (in Table 1) gel as in the duration study. The molecular weight of the polymer was studied as a function of glutathione concentration and time.

In-vitro Drug Release. Paclitaxel was dissolved in a poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer (P85-SS) aqueous solution (30.0 wt %) at a concentration of 2.5 mg/mL at 4 °C. All preparations were clear solutions. The polymer aqueous solution (0.5 mL) containing paclitaxel was injected through a 21-gauge syringe into a 4.0 mL vial (inner diameter = 12 mm), which was thermostated in a shaking water bath at 37 °C to form a gel. The total paclitaxel mass loaded was 1.25 mg. After 2 min, 3.0 mL of release medium (150 mM phosphate buffer saline with 0.1 wt % Tween 80, pH=7.4) at 37 °C was added to the preformed gel. The concentration of the glutathione in the release medium was varied from 0, 30, and 50 mM. The vial was shaken at 90 strokes/min. The release medium was replaced by a fresh one (3.0 mL) at designated sampling intervals. In addition, the glutathione concentration in the release medium was changed from 0 to 50 mM in 6 h to check the responsiveness of the release profile to the glutathione concentration.

The amount of released paclitaxel was determined by HPLC (Waters 600 with photodiode array detector at 227 nm) with a C18 reverse-phase column. The released paclitaxel concentration was calculated against the standard curve of paclitaxel in the release medium.

Results and Discussion

Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s (P65, P85, and P105) were selected for comparative purposes because they have the same weight percent of PEO (50 wt %) in the polymer. The hydroxyl end groups poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) were converted to thiol groups, followed by oxidative coupling of the thiol groups to prepare the disulfide multiblock copolymer (Figure 1). The triplet at 2.85 ppm in the ^{1}H NMR spectra ($OCH_2\boldsymbol{CH}_2-SS-$) and the peak at 14 min in the gel permeation chromatogram, where the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) appears at 16 min, indicate formation of the disulfide multiblock copolymer (Figure 2).

The molecular weight and molecular weight distribution of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s and their disulfide multiblock copolymers studied in this research are listed in Table 1. The molecular weight and molecular weight distribution of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer were 22 000−32 000 and 1.4−1.5, respectively. On the basis of the gel permeation chromatogram, the disulfide multiblock copolymers can be described as multiblock copolymers consisting of 3.6−4.4 poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s.

The phase diagrams of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s and their disulfide multiblock copoly-

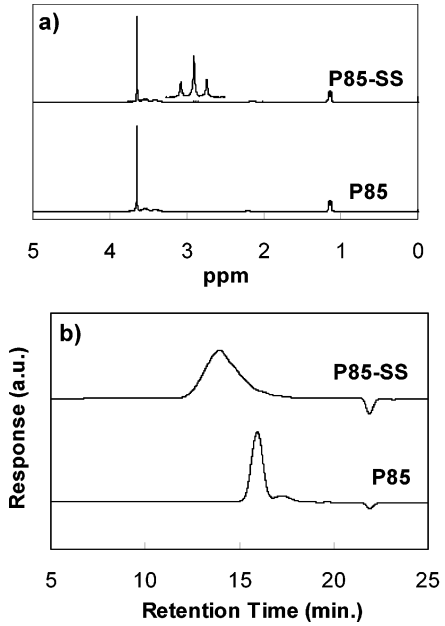

Figure 2. Follow up of the reactions: (a) ^{1}H NMR (in $CDCl_3$) of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P85) and the corresponding disulfide multiblock copolymer (P85-SS) and (b) gel permeation chromatogram of the P85 and P85-SS.

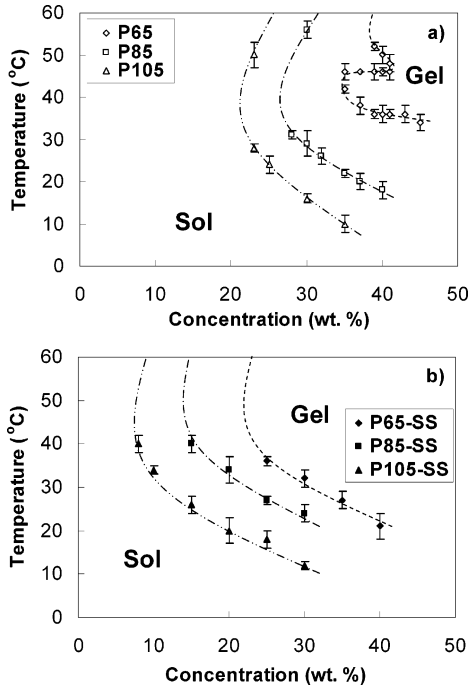

Figure 3. Phase diagram of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (a) and poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer (b) aqueous solutions determined by the test-tube inverting method. Each point is an average of three measurements.

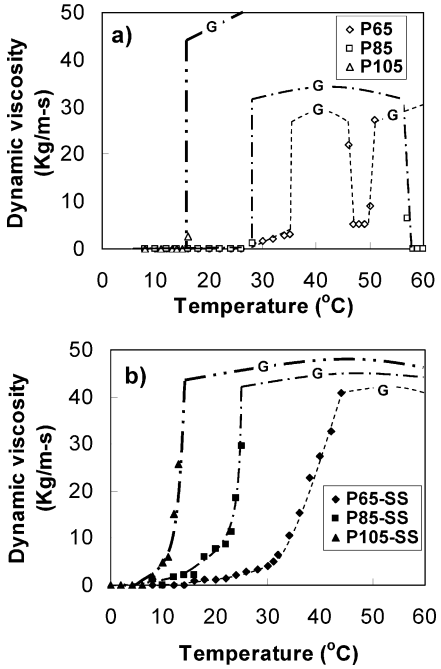

Figure 4. Dynamic viscosity of the (a) poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) and (b) disulfide multiblock copolymer aqueous solutions measured by the falling ball method. The sol−gel transition of the polymer aqueous solutions is seen by a vertical increase in the viscosity. All concentrations were 30 wt % except that P65, which was 40 wt %.

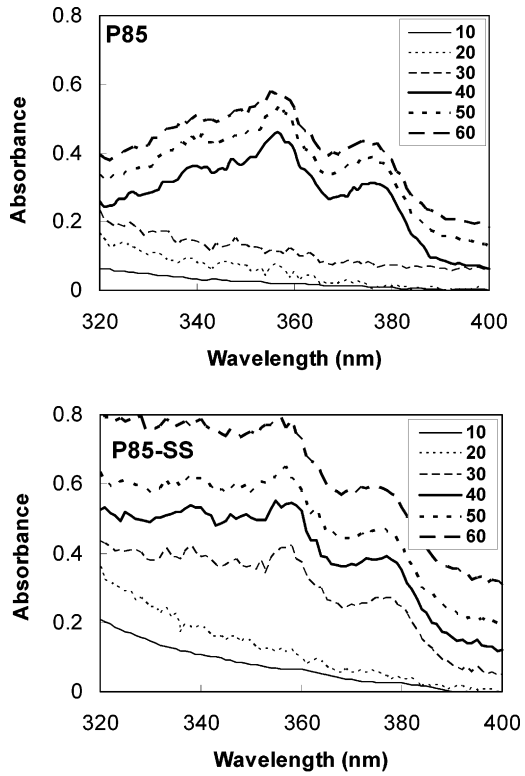

Figure 5. Dye solubilization in the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P85) and disulfide multiblock copolymer (P85-SS) aqueous solutions (0.5 wt %) as a function of temperature (legend).

mers were determined by the test-tube inverting method (Figure 3) in a temperature range of 0−60 °C. As the temperature increases, the P85 and P105 aqueous solutions showed sol−gel−sol whereas the P65 aqueous solution showed sol−gel−sol−gel transitions. The larger molecular weight P105 formed

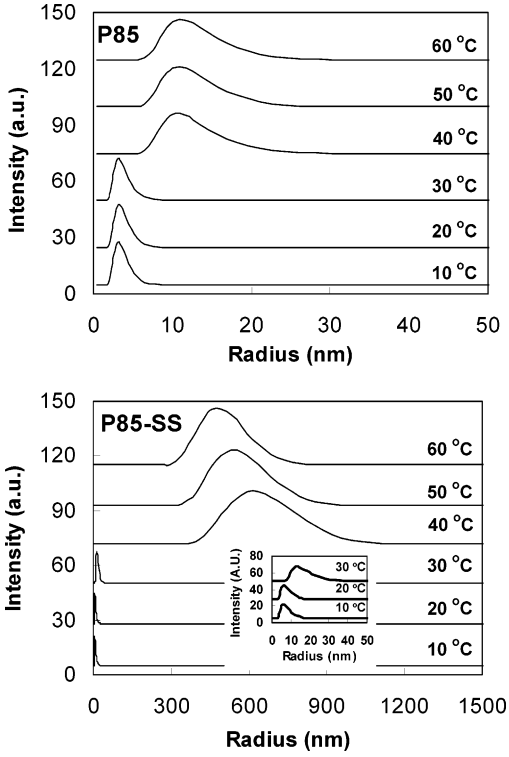

Figure 6. Apparent polymer size determined by dynamic light scattering of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P85) and disulfide multiblock copolymer (P85-SS) as a function of temperature at a concentration of 0.5 wt % in water. au indicates an arbitrary unit.

a gel at a lower concentration and the temperature to form a gel was lower compared with the smaller molecular weight P65. This fact suggests that the unimer-to-micelle transition is facilitated for a larger molecular weight polymer, as will be discussed later, even though they have the same weight ratio of PEO to PPO. Fluorescence study reported that the critical micelle temperature decreased as the molecular weight of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) increased at the same weight ratio of PEO to PPO.[33] The multiple sol−gel transition behavior was reported for other poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P103; $(EO)_{17}(PO)_{60}(EO)_{17}$) too. The low-temperature gel consists of spherical micelles close-packed in a cubic structure, whereas the high-temperature gel consists of rodlike micelles packed in a hexagonal structure.[34,35] Our current analysis shows that such a multiple transition behavior also depends on the total molecular weight of the polymer at the same composition.

On the contrary, the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer aqueous solution underwent sol−gel transition in the temperature range 0−60 °C. The disulfide multiblock copolymer (P105-SS) prepared from the larger molecular weight of poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P105) showed a lower sol−gel transition temperature than that prepared from the smaller molecular weight polymer (P65). The sol phase below 30 °C and the gel phase at 37 °C suggest their promising potential as an injectable carrier of pharmaceutical agents.

The falling ball method measures the transit time of a steel ball over a fixed distance at a given temperature. The steel ball dropped within a few seconds through the 4 cm sol, whereas it takes more than several minutes for the ball to drop through the gel phase. The steep increase in the transit time as a function of the temperature of the aqueous polymer solution the sol−gel transition temperature could be reproducibly determined.

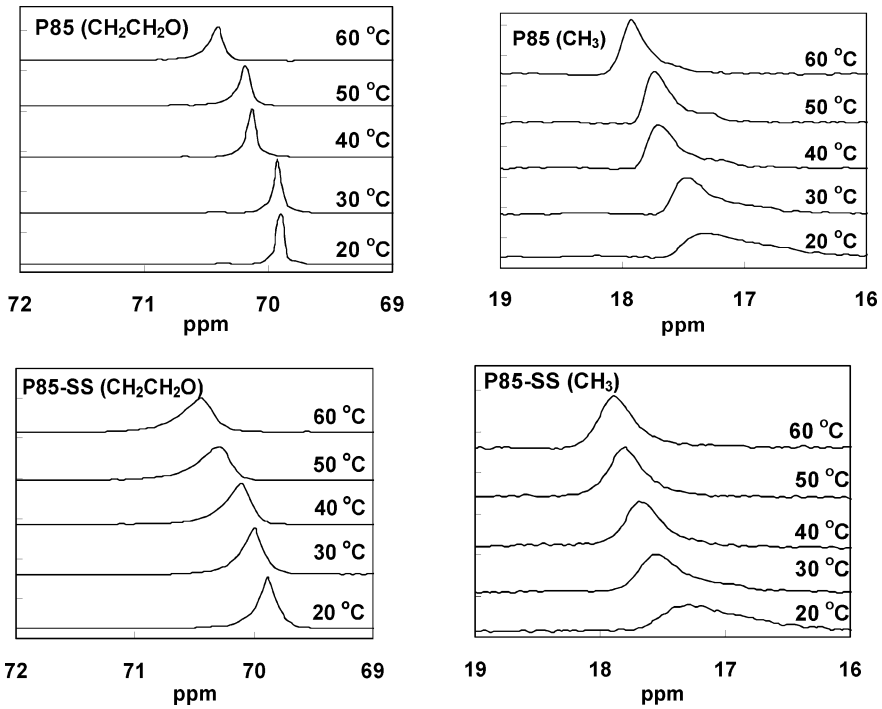

Figure 7. ^{13}C NMR spectra of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (P85) and disulfide multiblock copolymer (P85-SS) aqueous solutions (30 wt % in D_2O) as a function of temperature. The ethylene oxide group of PEO and the methyl group of PPO are shown.

The transition temperatures determined by the falling ball method were well correlated to those determined by the test-tube inverting method (Figure 4). The gel region is denoted as G in the graph. The P105 aqueous solution (30 wt %) showed only sol−gel transition as the temperature increased. However, the P85 aqueous solution (30 wt %) showed sol−gel−sol transitions. The P65 aqueous solution (40 wt %) showed a multiple sol−gel transition with a gel phase in 36−48 and 52−60 °C. On the contrary, the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer aqueous solutions showed only sol−gel transition by the falling ball method.

The sol−gel transition mechanism of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) at 35 °C is related to the unimer-to-micelle transition of the polymer as suggested by previous papers.[14] F127 undergoes unimer-to-micelle transition in water as the temperature increases, and the aqueous solution becomes a gel only when the micelle fraction is larger than 0.53.[36,37] The P85 aqueous solution (0.5 wt %) showed unimer-to-micelle transition at 35 °C as shown by the hydrophobic dye (1,6-diphenyl-1,3,5-hexatriene) solubilization method (Figure 5). The dye has a low absorptivity at 340, 355, and 378 nm in water; however, the absorptivity markedly increases in a hydrophobic environment. The increase in absorbance has been suggested as evidence of micelle formation as the temperature increases.[29,30] However, P85-SS showed an increase in the absorbance as well as an increase in the background scattering. This fact suggests formation of some larger particles of the poly-(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer as the temperature increases.

The dynamic light scattering study suggests that the P85 undergo unimer (3 nm) to micelle (12 nm) transition, whereas the corresponding disulfides multiblock copolymers (P85-SS) undergo aggregation (600 nm) of the unimer (6 nm) as the temperature increases (Figure 6). The increase in the absorbance of the P85 aqueous solution can be correlated to micelle formation, whereas the increase in the absorbance and scattering of P85-SS aqueous solution at the same time can be correlated to polymer aggregate formation. In addition, disulfide formation between thiol end groups of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer might contribute to the increase in the scattering intensity as will be discussed in the degradation study of the P85-SS. This suggests that the sol−gel transition mechanism at 30−35 °C might be related to the unimer (3 nm) to micelle (12 nm) transition for the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) whereas the unimer (6 nm) to aggregated polymer (600 nm) transition is involved for the corresponding disulfide multiblock copolymer.

^{13}C NMR study of the P85 and P85-SS aqueous solutions (30 wt %) suggests that both polymers underwent a similar change in that the PEO peak at 70 ppm was broadened whereas the PPO peak at 17 ppm was sharpened as the temperature increased (Figure 7). Both peaks underwent a downfield shift as the temperature increased. However, the chemical shifts in PEO and PPO peaks of P85 showed a stepwise transition at 30−40 and 50−60 °C, whereas those of P85-SS showed a steady change in a chemical shift as the temperature increased. These trends were correlated to the sol (20−30 °C)−gel (40−50 °C)−sol (60 °C) transition of the P85 and sol (20 °C)−gel (30−60 °C) transition of the P85-SS. The downfield shift was claimed as the change in hydration status of each block.[13,14] Dehydration of PEO and PPO seems to be involved as the temperature increases. However, the difference in the aggregate size of the polymer might result in the difference in the phase transition between the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) and its disulfide multiblock copolymer aqueous solutions.

One of the critical limitations of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) for biomedical applications is the short gel duration. The gel duration of the P85 was less than 6 h irrespective of the glutathione concentration in the medium. On the contrary, the disulfide multiblock copolymer gel lost less than 5% of its mass in phosphate buffer saline (pH 7.4) over 12 days. The longer duration comes from the difference in the gel morphology at 37 °C. The poly(ethylene

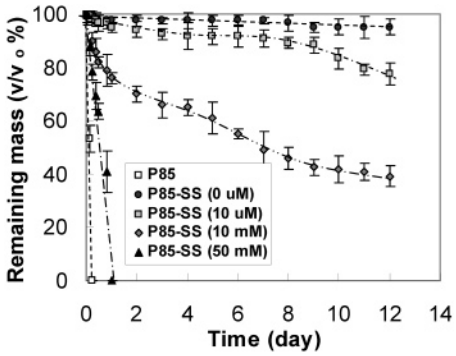

Figure 8. Duration of the in-situ-formed gel as a function of glutathione concentration in the medium. The initial concentration of the polymers was 30 wt %. Gel duration of P85 was independent of the glutathione concentration. The numbers in parentheses are the concentration of the glutathione in the medium. Each point is an average of three measurements.

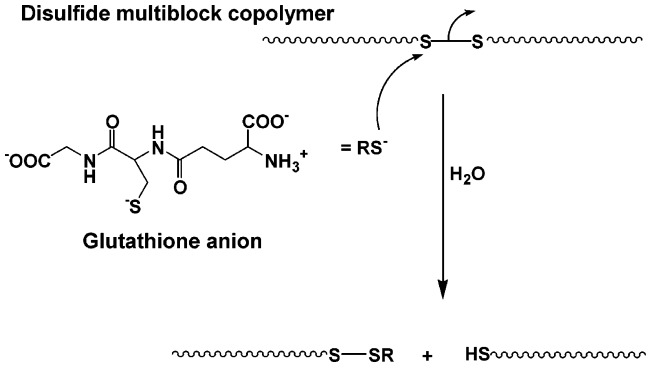

Figure 9. Schematic presentation of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer degradation by glutathione.

oxide-*b*-propylene oxide-*b*-ethylene oxide) gel has the micelle packed structure where the junctions are weakly connected through PEO blocks, whereas the disulfide multiblock copolymer can form intermicellar bridges (or polymer aggregates) at 37 °C.[14] Formation of particles with a 600 nm size as shown in dynamic light scattering as well as increases in the absorbance and background scattering in the dye solubilization study support this hypothesis. Such intermicellar bridges are more and more important at higher polymer concentration, resulting in an increase in the gel duration at 37 °C.

At 10 μM thiol concentration, a typical extracellular thiol concentration, the gel lost 20% of its mass over 12 days. At 10 mM thiol concentration, a typical intracellular thiol concentration, the gel lost 60% of its mass over the same period of time (Figure 8). At higher thiol concentration (50 mM) the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer lost its entire mass in a day. This indicates that degradation of the disulfide multiblock copolymer depends on the thiol concentration.

Degradation of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer by glutathione is schematically presented in Figure 9. The anion of thiol attacks the disulfide bond in a S_N2-type reaction. The polymer is cut into pieces, and one of the two end groups is substituted by the glutathione. Water acts as a proton donor or acceptor during the reaction.

The change in the gel permeation chromatogram of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer shows the degradation of the polymer as a function of thiol concentration (Figure 10a). During the

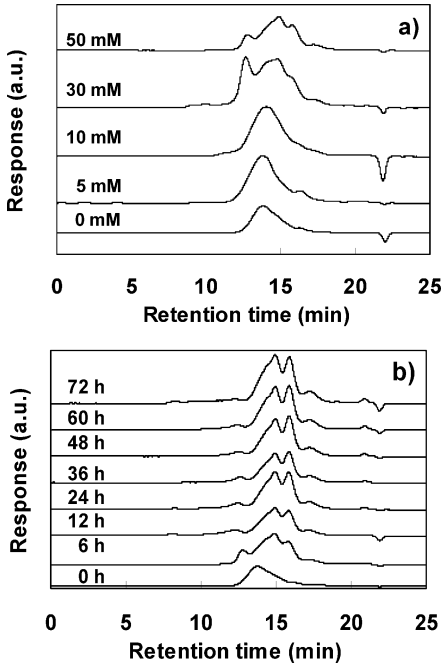

Figure 10. Degradation of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer (P85-SS) in 6 h as a function of glutathione (a) and as a function of time at a glutathione concentration of 50 mM (b).

degradation larger and smaller molecular weight peaks than the original polymer peak appear at the same time in the chromatogram. This suggests that some new bond formation between polymers and degradation occur at the same time. At pH 7.4, the thiol can be oxidized to disulfide because there is a significant amount of thiolate anions that are active form for thiol oxidation.[38] However, the dominant mode was the degradation. Degradation of the polymer as a function of time at a fixed thiol concentration (50 mM) is shown in Figure 10b. Similar trends of high and low molecular weight polymer formation were observed. However, the dominant mode was a decrease in the molecular weight of the polymer.

To confirm the drug release as a function of thiol concentration, the glutathione concentration of release medium was varied over 0−50 mM. As the glutathione concentration increased, the release rate became faster (Figure 11a). In the absence of the glutathione, less than 10% of paclitaxel was released in the experimental time period, whereas 60% of paclitaxel was released at a glutathione concentration of 50 mM over the same period. To see the responsiveness of the release profile on the amount of glutathione, the release medium without glutathione was replaced in 6 h by a new release medium containing 50 mM glutathione (Figure 11b). The consequent release of paclitaxel was observed. Therefore, thiol-sensitive drug release was confirmed.

Conclusions

The poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) aqueous solution underwent sol−gel−sol or sol−gel−sol−gel transition, whereas the corresponding disulfide multiblock copolymer aqueous solution underwent sol−gel transition in a temperature range of 0−60 °C. The phase diagrams of the three poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)s and the corresponding disulfide multiblock copolymer aqueous solutions were compared. However, other properties such as micellization, gelation, and degradation were focused on P85-SS because the other poly(ethylene oxide-*b*-propylene oxide-

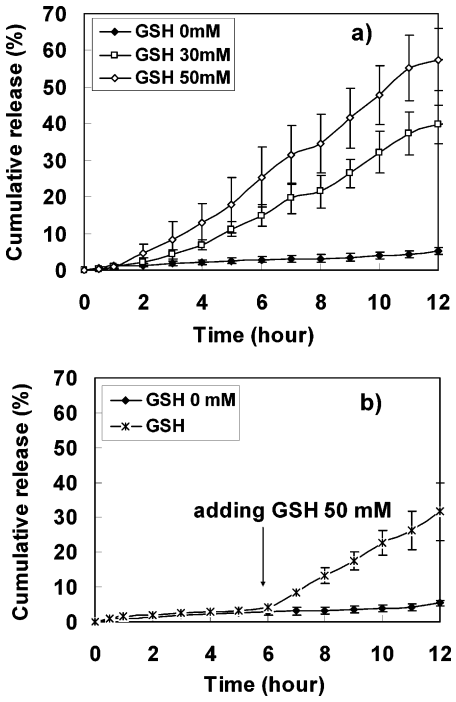

Figure 11. Release of taxol from the in-situ-formed poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer (P85-SS) gel (initial polymer concentration, 30 wt %) as a function of glutathione concentration in the release medium (a). The glutathione concentration was changed after 6 h to see the release of taxol in response to glutathione (b).

b-ethylene oxide) disulfide multiblock copolymers were assumed to show similar behavior.

The absorbance of hydrophobic dye sharply increased at 30−40 °C for poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) aqueous solution (0.5 wt %) reflecting the unimer-to-micelle transition, whereas that of the corresponding disulfide multiblock copolymer aqueous solution steadily increased with significant background scattering as the temperature increased. Dynamic light scattering of the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) aqueous solution suggested the unimer-to-micelle transition, whereas the disulfide multiblock copolymer showed unimer-to-aggregated polymer transition as the temperature increased. ^{13}C NMR showed that the two significant downfield shifts for both PEO and PPO were observed for the poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) that was correlated with multiple sol−gel transition, whereas the disulfide multiblock copolymer showed steady downfield shift of PEO and PPO as the temperature increased. Similar to other thermogelling polymers such as PEG/PLGA and PEG/PCL, the hydrophilic block (PEO) peak was broadened, however, the hydrophobic block (PPO) peak was sharpened.[7,12]

The in-situ-formed P85 gel was completely eroded in 6 h, and gel duration was not affected by glutathione concentration. On the contrary, the P85-SS gel persisted for more than 12 days in phosphate buffer saline, and gel duration was significantly affected by the glutathione concentration. The model drug, paclitaxel, was released from the in-situ-formed poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) disulfide multiblock copolymer gel in response to the change in glutathione concentration.

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