Sugar-Installed Polymer Micelles: Synthesis and Micellization of Poly(ethylene glycol)−Poly(D,L-lactide) Block Copolymers Having Sugar Groups at the PEG Chain End

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ABSTRACT: A poly(ethylene glycol)−poly(D,L-lactide) block copolymer (PEG−PLA) having a site specifically protected-sugar group at the PEG chain end was synthesized through a successive ring-opening polymerization of ethylene oxide and D,L-lactide using a metalated protected sugar as an initiator. Removal of protective groups from the sugar residue in the block copolymer was quantitatively carried out using 80% trifluoroacetic acid at room temperature, yielding a block copolymer having a glucose or galactose residue at the chain end in a regioselective manner. Polymer micelles having sugar residues on the surface were then prepared by dialyzing an *N*,*N*-dimethylacetamide solution of the sugar-bearing PEG−PLA block copolymer against water. Dynamic light-scattering measurement of the polymer micelle solution revealed that the scaled characteristics line width had essentially no angular dependence, consistent with the spherical geometry of the polymer micelle. The diameter and polydispersity index of the polymer micelle, determined by a cumulant method, were approximately 40 nm and less than 0.1, respectively. Further, a galactose-bearing PEG−PLA micelle was confirmed to selectively attach to RCA-1 lectin, which is known to recognize *β*-D-galactose residues. These polymer micelles having sugar groups regioselectively on their exterior are expected to have wide utility in the field of drug delivery as glyco-receptor-directed carrier systems.

Introduction

A notable property of AB block copolymers in a selective solvent is the self-association into a supramolecular assembly known as "polymer micelles".[1] Polymer micelles are in a mesoscopic size range (several tens of nanometers) and have a characteristic core−shell architecture, in which solvophilic segments of the block copolymer form an outer shell to surround the inner core composed of solvophobic segments.[2−4] The core may be utilized as a reservoir for solvophobic compounds, and on the other hand, the shell ensures the stable dispersity of the micelles in the solvent through a steric stabilization effect.[5] Because of their unique size and architecture, block copolymer micelles have attracted a growing interest in various fields as functionality nanofabricated materials.[6−17] In particular, in the field of drug delivery, extensive studies on amphiphilic block copolymer micelles have been carried out by many research groups including ours, proposing to use them as a novel carrier system for hydrophobic drugs.[18−25] Indeed, as reported in our previous paper,[23] micelle-formulated doxorubicin (a hydrophobic anticancer reagent), dosed through an intravenous route, retained longevity in blood circulation and eventually showed appreciable accumulation into a solid tumor due to the enhanced permeability of tumor vasculatures.

Further challenge in this fast-growing field of drug delivery is the development of micellar carrier systems for cellular-specific targeting in which pilot molecules are installed on the polymer micelle surface to achieve a specific binding property to target cells.[26,27] Of particular importance in this regard is the establishment of a novel and effective synthetic route to end-functionalized amphiphilic block copolymers with appreciable biocompatibility and biodegradability, allowing the conjugation of pilot molecules at the tethered end of the hydrophilic segment. Our research group has been carrying out a systematic study on the synthesis of end-functionalized block copolymers of biomedical interest,[28] including α-formyl-*ω*-hydroxyl (or α-formyl-*ω*-methacryloyl)−poly(ethylene glycol)-*block*-poly(D,L-lactide) (α-formyl-*ω*-hydroxyl- or α-formyl-*ω*-methacryloyl−PEG−PLA).[14,29]

Here, we report a novel one-pot synthesis and subsequent micellization in aqueous milieu of PEG−PLA block copolymers having particular sugar groups, including glucose and galactose, at the PEG chain end in a regioselective manner. As well-documented in the literature,[30−32] sugars play a crucial role in the process of cell-involved biorecognition, and indeed, sugar-mediated drug delivery to target cells via glyco-receptors on the cellular plasma membrane is expected to be one of the most promising routes in cellular-specific drug targeting.[33−35] It should be noted, however, that glyco-receptor binding to a particular sugar often occurs in a regioselective manner.[36−38] The linkage of sugar to the polymer is generally formed by glycosyl or 1-O substitution because of the convenient installation of the sugar into the polymer chain.[39−43] However, the linkage at other positions as C-6 and C-2 is also available in the literature for the sugar installation. For example, Kopecek and Duncan reported that the *N*-(2-hydroxy-

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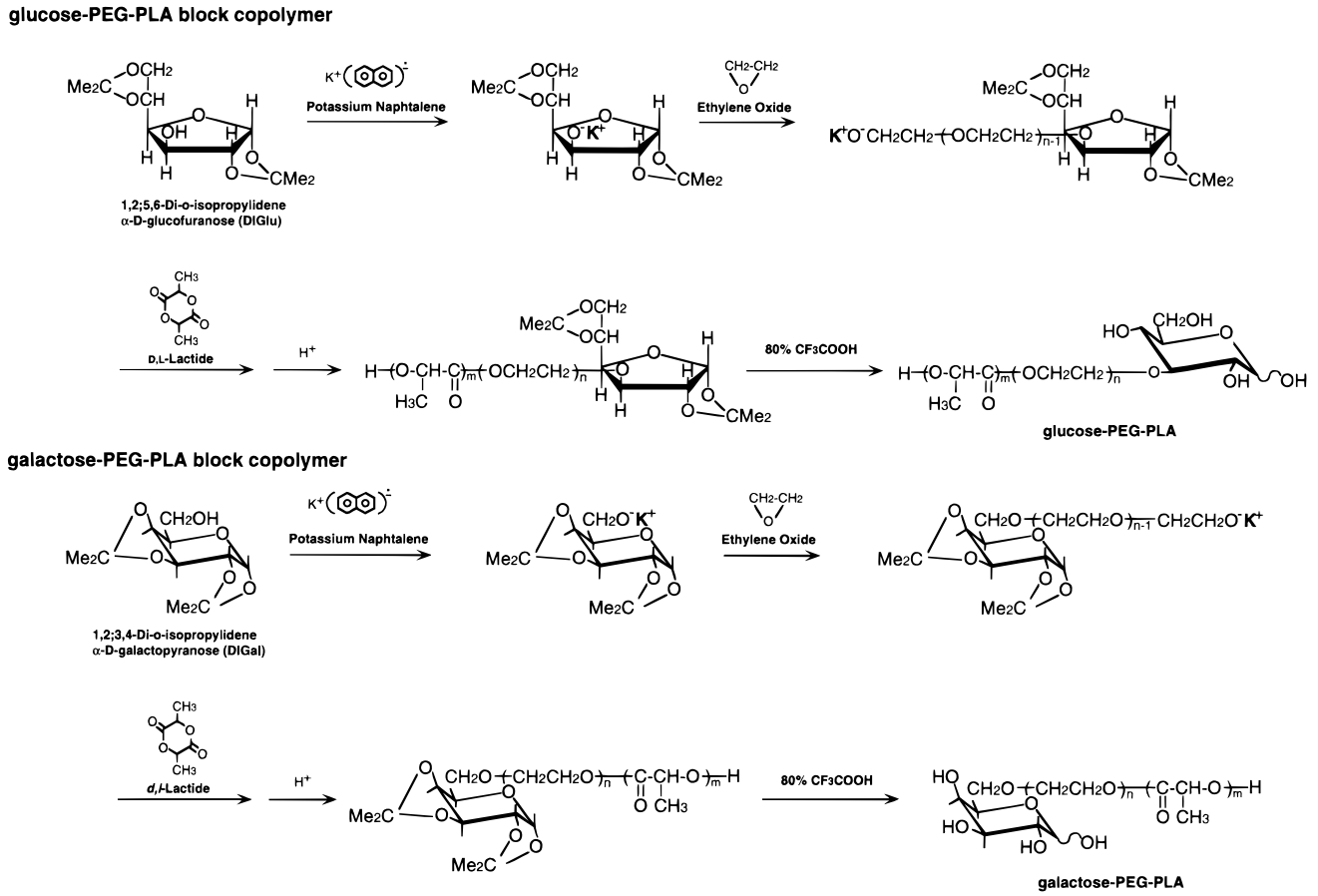
Scheme 1. Synthetic Route of Sugar−PEG−PLA Block Copolymers by Anionic Ring-Opening Polymerization.



propyl) methacrylamide(HPMA) copolymers containing galactose linked at the C-6 position had an effective targeting property to the liver, which was proved from the study on the body distribution of radiolabeled polymers after iv administration to rats.[44] It was also found that appreciable delivery of the polymer-conjugated drug to the liver was performed by the HPMA with galactosamine linked at the C-2 position.[45] Further, the reduced end of glucose is essential for the recognition of glucose-derivatized polymers by glucose transporter.[46,47] These examples indicate that regioselective introduction of the sugar to the polymer through a substitution other than 1-O substitution is also practical for constructing the sugar-targeted polymeric drug system. Thus, in this regard, the regioselective introduction of sugar groups at the chain end of the block copolymer is urgently needed. This was successfully achieved in this study by utilizing a metal alkoxide of a site-specifically protected sugar as an initiator of the anionic ring-opening polymerization of ethylene oxide.

Experimental Section

Materials. Ethylene oxide (EO) (3M Healthcare Co., Ltd.) was purified by distillation under CaH_2. D,L-Lactide (Tokyo Chemical Industry Co., Ltd.) was recrystallized twice from ethyl acetate, followed by sublimation under vacuum at 110 °C. Tetrahydrofuran (THF) was purified conventionally.[48] *N*,*N*-Dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMAc), ethyl acetate, trifluoroacetic acid (TFA), 2-propanol, ligroin, and naphthalene were purchased from Wako Pure Chemical Industries, Ltd. and used without further purification. Water was purified either by Labo IonPure-12 (Millipore Corporation) or by a Milli-Q SP Reagent Water System (Millipore Corporation). 1,2;5,6-di-*O*-isopropylidene-α-D-glucopyranose (DIGlu) (Pfanstiehl, Ltd.) and 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose (DIGal) (Pfanstiehl, Ltd.) were purified, respectively, by recrystallization from ligroin and by distillation under vacuum (bp = 154 °C at 4 mmHg). A THF solution of potassium naphthalene was prepared by adding 45.5 mmol (1.77 g) of freshly cut potassium (Wako Pure Chemical Industries, Ltd.) into 100 mL of ice-cooled THF solution including 35 mmol (4.49 g) of naphthalene.[49] After 24 h of stirring at room temperature, unreacted potassium in the solution was removed by decantation. The concentration of potassium naphthalene thus prepared was determined by titration.

Synthesis of PEG−PLA Block Copolymers Having a Protected-Sugar Group at the PEG Chain End. PEG−PLA block copolymers having a protected-sugar group at the PEG chain end were synthesized by successive anionic ring-opening polymerization of EO and D,L-lactide at room temperature under argon atmosphere as shown in Scheme 1, using a metalated protected-sugar as an initiator. A typical procedure is as follows: 0.5 mmol of potassium naphthalene in 1.8 mL of THF was added to 20 mL of dry THF including 0.5 mmol of DIGlu to prepare the initiator, metalated DIGlu (K−DIGlu). After this mixture was stirred for a few minutes, 57 mmol (2.85 mL) of EO was added via a cooled syringe to the THF solution containing K−DIGlu. The polymerization reaction of EO was carried out at room temperature for 2 days, resulting in formation of a highly viscous solution. After a small portion of the solution was sampled via a syringe for the analysis of molecular weight and distribution of PEG thus prepared, 20.8 mmol (25 mL) of a D,L-lactide solution in THF (8.3mol/L) was introduced into the reaction vessel, and the reaction was continued at room temperature for another 4 h. The mixture was then precipitated into a 30-fold excess of ice-cold 2-propanol. Precipitated polymer was collected by centrifugation for

30 min at 2010*g* and then was redissolved in benzene for lyophilization. The freeze-dried sample as a white powder was stored at −20 °C until use.

Characterization of Block Copolymers. The molecular weight and the distribution (M_w/M_n) of sampling PEG and the block copolymer were determined by gel permeation chromatography (GPC). GPC measurements were performed at 40 °C at a flow rate of 0.8 mL/min using a liquid chromatograph (JASCO, Japan) equipped with TSK gel columns ($G4000H_{HR}$ + $G3000H_{HR}$) (Tosoh Co., Ltd.) and an internal refractive index (RI) detector (930-RI, JASCO). DMF containing 10 mmol LiCl was used as an eluent. Molecular weight calibration was done using a series of standard PEGs (Polymer Laboratories) with varying molecular weights ranging between 440 and 23 000. Samples were dissolved in the eluent at a concentration range of 2−5 mg/mL and were injected into the GPC circuit through a 100 μL loop.

To determine the composition of the block copolymer, 400 MHz ^{1}H NMR measurements (JEOL EX400, Japan) were carried out in dimethyl-d_6 sulfoxide (DMSO-d_6) at 60 °C. The molecular weight of the PLA segment in the block copolymer was estimated from a ^{1}H NMR spectrum by examining the peak intensity ratio of the methine proton of the PLA segment ($COCH(CH_3)O$: δ 5.2 ppm) and the methylene protons of the PEG segment (OCH_2CH_2: δ 3.6 ppm) based on the number-averaged molecular weight (M_n) of PEG determined from the GPC measurement. The functionality of the sugar was also estimated from a ^{1}H NMR spectrum by examining the intensity ratio of H-1 proton of the protected-sugar or anomeric proton of the deprotected sugar and the methylene protons of PEG segment (OCH_2CH_2: δ 3.6 ppm) based on M_n of PEG determined from the GPC measurement.

Removal of Protective Groups from Sugar Residues in the Block Copolymer. Deprotection of the sugar residue in the block copolymer was performed in a mixture of trifluoroacetic acid (TFA)−water of varying composition (TFA:water (v/v) = 9:1, 8:2, 7:3) for a definite time period at room temperature. The reaction was stopped by pouring the reaction mixture into a 50-fold excess of 2-propanol cooled to −20 °C. The precipitate was collected and redissolved in 2-propanol followed by dialysis against water using a semipermeable membrane (Spectra/Por; cutoff molecular weight, 1000). The dialysate was exchanged at 2, 5, and 8 h from the beginning of dialysis. Block copolymers with the deprotected-sugar group were obtained as white powders by lyophilization after the dialysis.

Preparation of Polymer Micelles. Micelle preparation was done following the method detailed in our previous publications.[14,26] Briefly, 50 mg of the copolymer was dissolved in 10 mL of DMAc. The solution was then transferred through a 0.45 μm filter (Millex FH_{13}, Millipore Corporation) into a preswollen semipermeable membrane (Spectra/Por; cutoff molecular weight, 3500) and was dialyzed against 1 L of water for 24 h. The dialysate was exchanged at 2, 5, and 8 h from the beginning of the dialysis.

Dynamic Light-Scattering Measurement. Hydrodynamic radii and polydispersity indices of the polymer micelles were estimated by dynamic light scattering (DLS) at 25 °C, using a light-scattering spectrophotometer (DLS-7000, Photal, Otsuka Electronics) with a vertically polarized incident beam at 488 nm supplied by an argon ion laser. A scattering angular range of 30−150° was used in this study.

In the DLS measurements, the general formula for the photoelectron count time correlation function has the form

$$g^{(2)}(\tau) = 1 + \beta|g^{(1)}(\tau)|^2 = 1 + \beta \exp(-2\bar{\Gamma}\tau) \qquad (1)$$

where $g^{(2)}(\tau)$ is the normalized second-order correlation function, β is a parameter of the optical system, $g^{(1)}(\tau)$ is the normalized first-order correlation function, τ is the delay time, and $\bar{\Gamma}$ is the average characteristic line width. In the cumulant approach, $g^{(1)}(\tau)$ can be expressed by the following equation:

$$g^{(1)}(\tau) = \exp[-\bar{\Gamma}\tau + (\mu_2/2!)\tau^2 - (\mu_3/3!)\tau^3 + ...] \qquad (2)$$

This yields $\bar{\Gamma}$ and the variance (polydispersity index), $\mu_2/\bar{\Gamma}^2$. The *z*-averaged diffusion coefficient, D, and hydrodynamic diameter, d, can be obtained from $\bar{\Gamma}$ using the following equations:

$$\bar{\Gamma} = DK^2 = D[(4n_0/\lambda_0)\sin(\theta/2)]^2 \qquad (3)$$

$$d = k_B T/(3\eta_0 D) \quad \text{(Stokes−Einstein equation)} \qquad (4)$$

Here η_0 is the refractive index of the solvent, λ_0 is the wavelength, and θ is the scattering angle. The size distribution was also estimated from the correlation function profile using the histogram method.[50]

Interaction between the Sugar-Bearing Micelle and RCA-1 Lectin. Pyrene-loading into sugar-bearing polymer micelles was carried out based on the procedure described eleswhere.[26,29,51] Interaction between pyrene-loaded polymer micelles and *Ricinus communis* lectin (RCA-1 lectin) was explored by passing the polymer micelle solution into a column packed with RCA-1 immobilized beads (Shodex AFpak ARC-894, Showa Denko K. K.). Measurements were performed at room temperature at a flow rate of 0.8 mL/min using a liquid chromatograph (JASCO, Japan) equipped with the RCA-1 immobilized column and a fluorescence detector (820-FP, JASCO, Japan) (E_x = 339 nm, E_m = 375 nm). A $^1/_{15}$ M phosphate buffer (pH 7.4) containing 0.15 M NaCl was used as an eluent. The sample was injected into the column circuit through a 4 μL loop.

Results and Discussion

Synthesis of PEG−PLA Block Copolymers Having a Protected-Sugar Group at the PEG Chain End. As we reported previously, a heterobifunctional PEG with a sugar residue at the α-end can be synthesized by anionic ring-opening polymerization of EO using the potassium alkoxide of a site-specifically protected sugar molecule as an initiator.[52] Here, we extended this method to the regio-selective introduction of a sugar group at the chain end of PEG−PLA block copolymers. For this purpose, a site-specifically metalated protected-sugar was used as an initiator of block copolymerization as shown in Scheme 1. The polymerization was initiated at the C-3 position of glucose and the C-6 position of galactose for DIGlu and DIGal, respectively. In this procedure, D,L-lactide polymerization was immediately succeeded by EO polymerization in the same reaction vessel to obtain the PEG−PLA block copolymer having a protected-sugar group at the α-end of the PEG chain end. After the step of ring-opening polymerization of EO, the produced PEG still has an active potassium alkoxide moiety at the ω-end. Strong nucleophiles such as this potassium alkoxide can attack the lactide and induce O═C−O bond cleavage leading to the formation of a potassium alkoxide propagating species.[53] Eventually, the ring-opening polymerization of lactide is initiated from the ω-end of PEG.

Figure 1 shows the gel permeation chromatograms of the DIGlu−PEG and DIGlu−PEG−PLA block copolymer thus prepared. The M_n of DIGlu−PEG, sampled after the first stage of polymerization, was determined to be 5400 by GPC, which is in good accordance with the calculated value, M_n = 5276, based on a monomer-to-initiator ratio. It was also obvious from the chromatogram that the molecular weight had been increased after the stage of D,L-lactide polymerization without broadening of the distribution (M_w/M_n = 1.10). This result indicates that the block copolymerization proceeds almost quantitatively utilizing K−DIGlu as an initiator. A similar result was obtained when K−DIGal was used as an initiator.

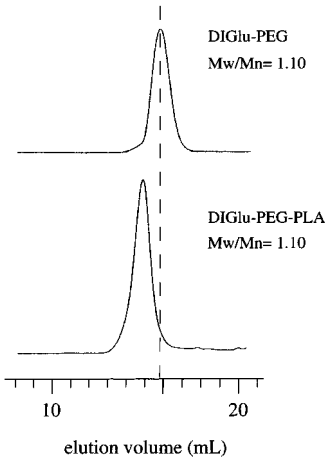


Figure 1. Gel permeation chromatograms of DIGlu−PEG and DIGlu−PEG−PLA(54−58) block copolymers. (Temperature, 40 °C; eluent, DMF containing 10 mM LiCl).

Both the composition of the block copolymers and the molecular weight of the PLA segment in the block copolymer were estimated from the ^{1}H NMR spectra (Figures 2 and 3). All the peaks in the ^{1}H NMR spectra were assignable to protons in the assumed structure of the block copolymers. The assignment was performed by reference to the previous papers.[54−58] M_n of the PLA segment was then calculated using the peak intensity ratio of methylene protons of PEG and the methine proton of PLA in the ^{1}H NMR spectra based on the M_n of PEG determined by GPC.

Characterized values, including the molecular weight and its distribution, are summarized in Table 1. The abbreviation $X-Y$ was used to express the composition of the block copolymers, where X stands for $M_n \times 10^{-2}$ of PEG and Y for $M_n \times 10^{-2}$ of PLA segment. Both DIGlu−PEG−PLA and DIGal−PEG−PLA block copolymers were confirmed to have a sufficiently narrow molecular weight distribution ($M_w/M_n = 1.10$).

The ^{1}H NMR spectra of DIGlu− and DIGal−PEG−PLA block copolymers shown in Figures 2 and 3 also provide information on the structure of the sugar residue located at the α-end of the block copolymers. The signals appearing between 3.5 and 6.0 ppm in the ^{1}H NMR spectrum of the DIGlu−PEG−PLA block copolymer (Figure 2) can be attributed to the protons on the glucofuranosyl derivative: δ 5.82 (d, 1H, DIGlu H-1, $J_{12} = 3.7$ Hz), 4.58 (d, 1H, DIGlu H-2, $J_{12} = 3.8$ Hz), 4.22 (m, 4H, DIGlu H-5 + CH of PLA segment and CH_2 of PEG segment (*d*)), 4.08 (dd, 1H DIGlu H-4, $J_{34} = 2.9$, $J_{45} = 6.3$ Hz), 3.97 (dd, 1H DIGlu H-6b, $J_{56b} = 4.7$, $J_{6a6b} = 6.8$ Hz), 3.88 (d, 1H DIGlu H-3, $J_{34} = 2.9$ Hz), and 3.81 (dd, 1H DIGlu H-6a, $J_{56a} = 5.2$, $J_{6a6b} = 6.8$ Hz). Similarly, the signals appearing in the range 3.8−5.5 ppm in the ^{1}H NMR spectrum of the DIGal−PEG−PLA block copolymer (Figure 3) can be attributed to the protons on the galactopyranosyl derivative: δ 5.44 (d, 1H, DIGal H-1, $J_{12} = 5.3$ Hz), 4.56 (dd, 1H, DIGal H-3, $J_{23} = 2.1$, $J_{34} = 7.5$ Hz), 4.30 (dd, 1H DIGal H-2, $J_{12} = 5.2$, $J_{23} = 2.1$ Hz), 4.20 (m, 2H DIGal H-4 + CH of PLA segment and CH_2 of PEG segment (*d*)), and 3.85 (m, 1H DIGal H-5). Methyl protons in the protective groups clearly appeared as peaks centered at 1.3 ppm, demonstrating that the block copolymers have a protected-sugar group.

Removal of Protective Groups from Sugar Residues in the Block Copolymer. Removal of isopropylidene groups from the sugar residue at the α-end of the block copolymer was carried out using of 80% trifluoroacetic acid (TFA) at room temperature. TFA treatment is the general procedure to remove protective groups such as an isopropylidene unit from the sugar residue.[59] It should be noted that the hydrolysis of ether linkage in PEG as well as that settled between PEG and the sugar moiety is not occurred by this TFA treatment,[59−61] yet special care in the reaction conditions was needed because of the high susceptibility of ester linkages in the PLA segment toward acid hydrolysis. Indeed, as shown in run 1 in Table 2, treatment with 90% TFA for 20 min caused significant hydrolysis of the PLA segment, resulting in a widening of the molecular weight distribution of the block copolymers. On the other hand, neither removal of the isopropylidene groups nor hydrolysis of the PLA was accomplished by treatment with 70% TFA even with a prolonged reaction period of 60 min (run 3 in Table 2). Thus, 80% TFA treatment for

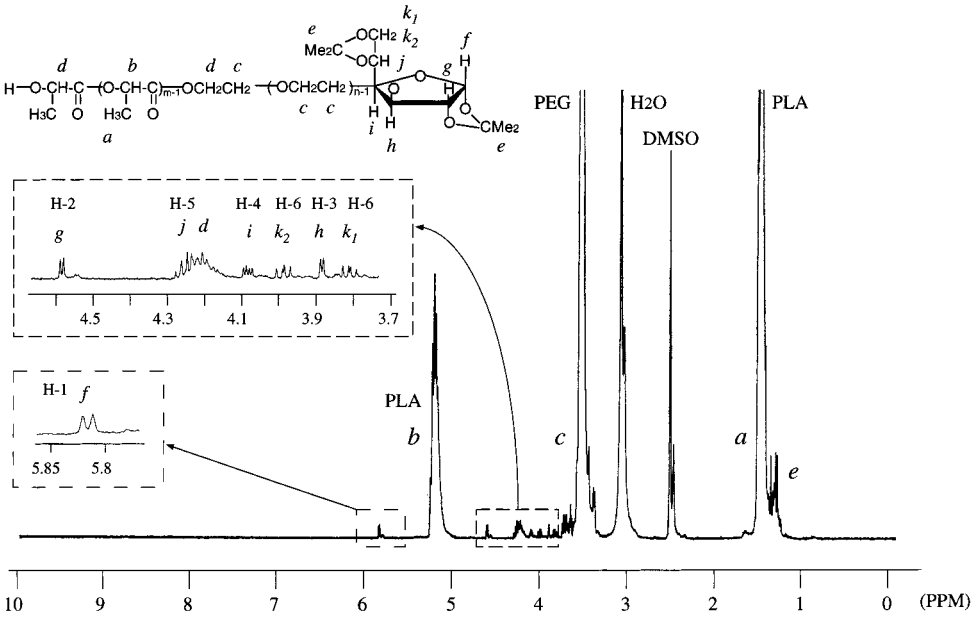


Figure 2. ^{1}H NMR spectrum of DIGlu−PEG−PLA(54−58) block copolymer in DMSO-d_6 at 60 °C.

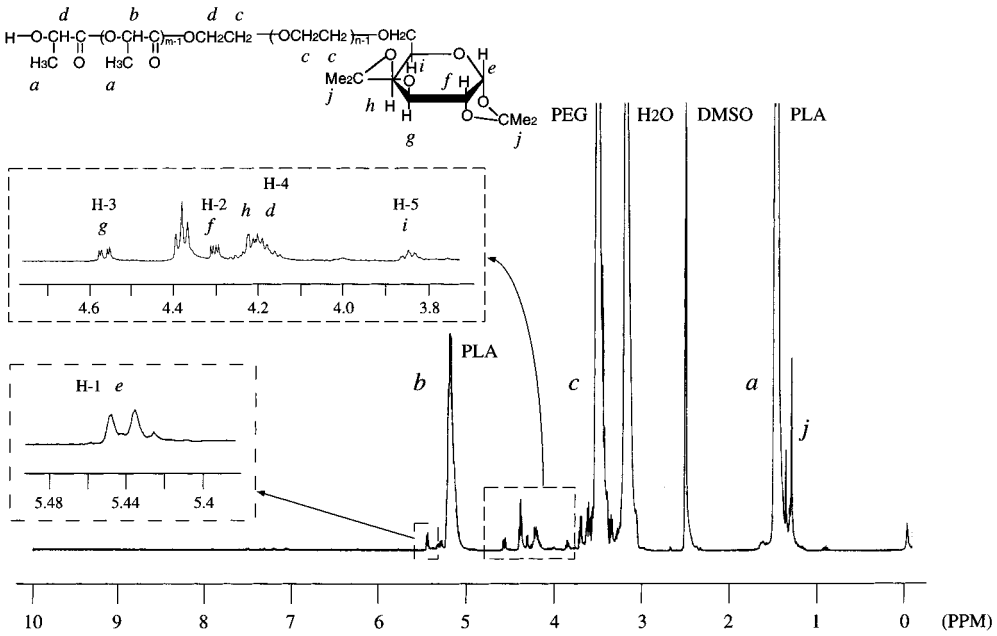


Figure 3. ^{1}H NMR spectrum of DIGal−PEG−PLA(61−35) block copolymer in DMSO-d_6 at 60 °C.

Table 1. Characteristics of DIGlu−PEG−PLA and DIGal−PEG−PLA Block Copolymers

polymer	M_n of PEG[a] (g/mol)	M_w/M_n of PEG[a]	M_n of PLA[b] (g/mol)	M_w/M_n of the block copolymer[a]	weight ratio of PLA to PEG	yield (%)
DIGlu−PEG−PLA(54−58)	5400	1.10	5800	1.10	1.07	75.5
DIGal−PEG−PLA(61−35)	6100	1.04	3500	1.09	0.57	62.0

[a] Determined by GPC measurement. [b] Determined by 1H NMR measurement in DMSO-d_6 at 60 °C.

Table 2. Removal of the Protective Group from DIGlu−PEG−PLA (54−58) by TFA

run	H_2O:CF_3COOH (v:v)	reaction time (min)	M_n of PLA	M_w/M_n	ratio of H-1 on DIGlu to anomeric proton of glucose[a]
1	1:9	20	4440	1.31	0.17
2	2:8	30	4810	1.17	0
3	3:7	60	4780	1.11	0.60

[a] The ratio was calculated using the peak intensity of the H-1 of DIGlu and anomeric proton of glucose in ^{1}H NMR spectrum based on the intensity of methylene protons of PEG.

30 min (run 2 in Table 2) was chosen as the optimum condition in this study to remove protective groups from the sugar residue even though concomitant hydrolysis of the PLA segment may occur to a slight extent.

Figure 4 shows the GPC data demonstrating the change in the molecular weight and the distribution of the block copolymer with 80% TFA treatment under the most suitable conditions (Run 2 in Table 2). Only a slight decrease was observed in the molecular weight as well as a slight increase in the molecular weight distribution (1.10 to 1.17) after 80% TFA treatment. An average decrease in the polymerization degree of the PLA segment under this reaction condition was estimated to be 16 units, corresponding to $M_n = 1200$, from the ^{1}H NMR spectrum as summarized in Table 3, indicating that 77.8% of the PLA units tolerate the acid treatment. Note that the distribution of the block copolymers remained unimodal with a considerably narrow distribution even after TFA treatment, suggesting that the degradation may proceed from the PLA chain end (exodegradation) rather than by random scission (endodegradation) mechanism.

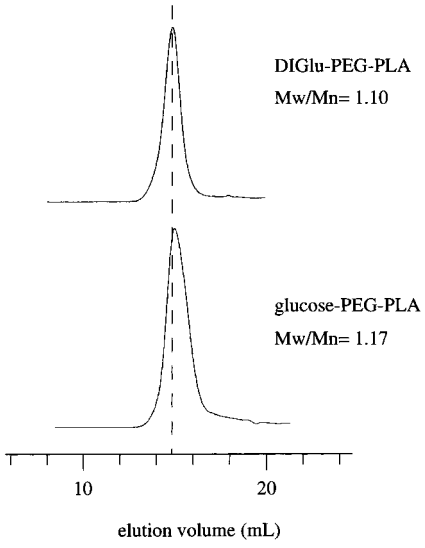


Figure 4. Gel Permeation Chromatograms of DIGlu−PEG−PLA(54−58) and glucose−PEG−PLA(54−48) block copolymers. (Temperature, 40 °C; eluent, DMF containing 10 mM LiCl)

It should be noted that, as reported in our pervious paper, the molecular weight distribution of the block copolymer is the key determining factor of the dispersity of the micelle formed through the association of the block copolymer,[51] and the considerably narrow molecular weight distribution of the sugar−PEG−PLA block copolymer prepared in this procedure is preferable for the micelle preparation, which is discussed in detail in the following section.

The peaks in the ^{1}H NMR spectrum of glucose−PEG−PLA block copolymer obtained by 80% TFA treatment

Table 3. Change in the Molecular Weight of PLA Segment and Sugar Functionality by 80% TFA Treatment

polymer	TFA treatment	M_n of PLA (g/mol)	M_w/M_n of the block copolymer	weight ratio of PLA to PEG	functionality[a] (%)
DIGlu−PEG−PLA(54−58)	before	5800	1.10	1.07	90.8
	after	4800	1.17	0.89	88.6
DIGal−PEG−PLA(61−35)	before	3500	1.08	0.57	91.7
	after	2200	1.07	0.36	90.3

[a] The functionality of the sugar was estimated from the ratio of H-1 proton of the protected-sugar or anomeric proton of deprotected-sugar and methylene protons of PEG

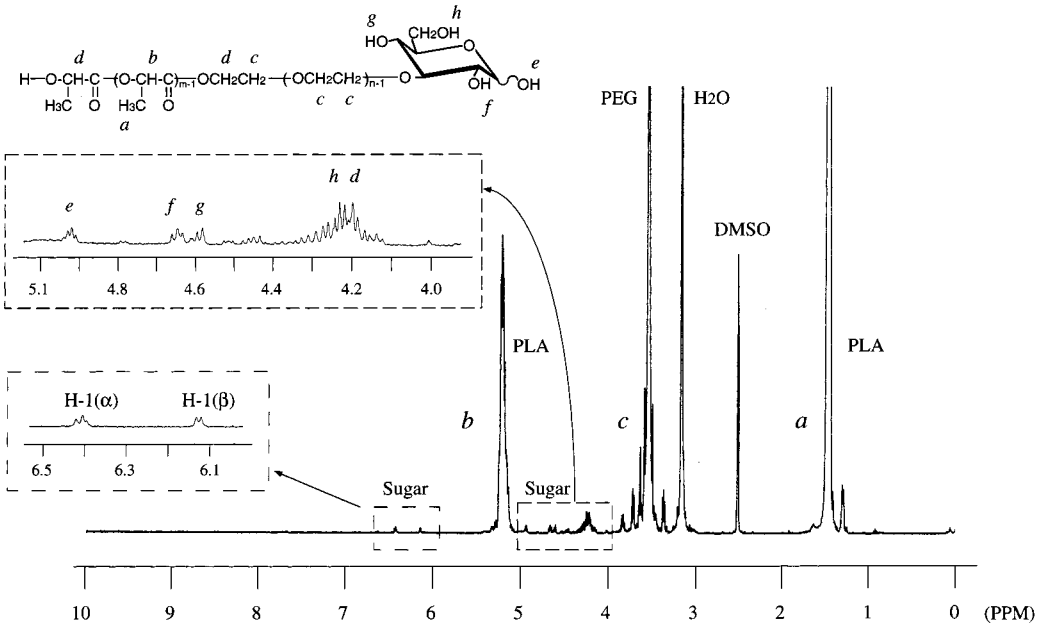


Figure 5. ^{1}H NMR spectrum of glucose−PEG−PLA(54−48) block copolymer in DMSO-d_6 at 60 °C.

was consistently assigned to the assumed structure as shown in Figure 5. The peaks were assigned on the basis of the data given in references 55, 56, and 58. Obviously, the peaks of the methylene protons (δ 1.3 ppm) from the isopropylidene groups and of H-1 (δ 5.82 ppm) on the protected glucopyranosyl derivative disappeared after acid treatment, and on the other hand, anomeric protons at the C-1 position (reduced end) of glucopyranose clearly appeared at 6.13 and 6.42 ppm, which can be assigned to H-1$_\beta$, ($J_{1\beta2}$ = 4.3 Hz) and H-1$_\alpha$ ($J_{1\alpha2}$ = 6.4 Hz), respectively. Peaks of the OH groups in the glucopyranosyl unit appeared in the range 4.1−5.0 ppm. Consequently, the ^{1}H NMR spectrum clearly confirmed the complete conversion of DIGlu to glucose within 30 min under the reaction conditions of run 2 in Table 2.

Removal of the protection groups was clearly confirmed also for the galactose−PEG−PLA block copolymer as shown in Figure 6. Peaks from the isopropylidene groups (δ 1.3 ppm) and of H-1 (δ 5.44 ppm) on the protected galactopyranosyl derivative disappeared completely after acid treatment. In line with the disappearance of peaks corresponding to the protective groups, new peaks appeared corresponding to anomeric protons (δ 5.99 ppm, $J_{1\beta2}$ = 4.5 Hz, δ 6.34 ppm, $J_{1\alpha2}$ = 6.3 Hz) as well as hydroxyl protons (δ 4.38 ppm) from the galactopyranosyl unit. The conversion of DIGal to galactose was completed within 30 min as was the case with DIGlu conversion to glucose.

The functionality of sugar before and after the deprotection is also summarized in Table 3. High functionality was observed in both protected and deprotected sugar−PEG−PLA block copolymers, indicating that protected sugar was quantitatively converted to the corresponding sugar without the release or hydrolysis of the sugar moiety by TFA treatment.

Preparation and Characterization of Polymer Micelles with Sugar Groups on Their Surface. Block copolymer micelles are usually prepared by changing the composition of the solvent in the way that satisfies the condition of a selective solvent for either of the blocks.[26,62] In this study, dialysis was used for solvent exchange to obtain polymer micelles stably dispersed in an aqueous medium.

The size and the dispersity of the polymer micelle obtained by dialysis were estimated by DLS. To obtain information on the shape and dispersity of the polymer micelle, angle-trace DLS measurements were carried out at detection angles of 30, 60, 90, 120, and 150°. For spherical particles, the scaled characteristic line width ($\bar{\Gamma}/K^2$) should be independent of the detection angle due to the undetectable rotational motion. Figure 7 shows the result of the angle-trace DLS measurement of the glucose−PEG−PLA micelle. Obviously, $\bar{\Gamma}/K^2$ had no angular dependence, which is consistent with the spherical geometry. The normalized coefficient of τ^2, $\mu_2/\bar{\Gamma}^2$, in eq 2 in the Experimental Section is called the polydispersity index (PDI) and indicates the degree of the dispersity of the prepared polymer micelles.[63] The values of the PDI determined at different detection angles were always below 0.1, indicating that the system is regarded as essentially monodispersive without formation of larger aggregates or a cluster of micelles.

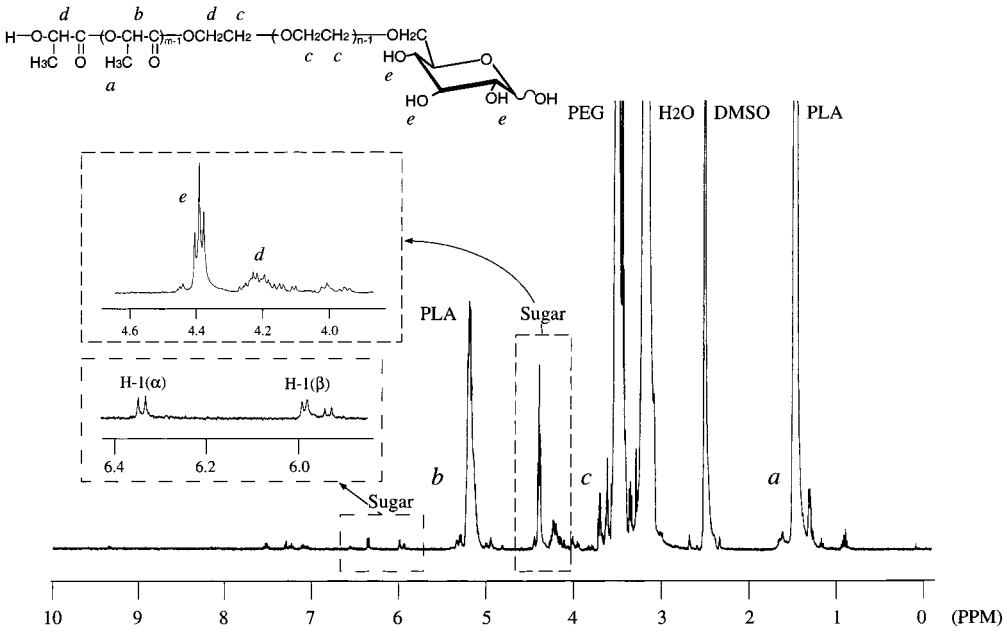


Figure 6. ^{1}H NMR spectrum of galactose−PEG−PLA(61−22) block copolymer in DMSO-d_6 at 60 °C.

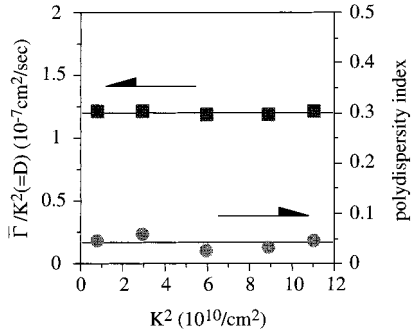


Figure 7. Change in the scaled average characteristic line width ($\bar{\Gamma}/K^2$) and polydispersity index with the magnitude of the scattering vector (K^2) of the glucose−PEG−PLA(54−48) micelle. The data points are as follows: (■) $\bar{\Gamma}/K^2$; (●) polydispersity index. (Detection angle: 30−150°.)

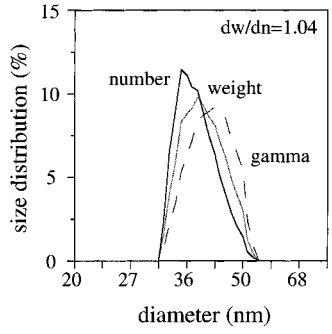


Figure 8. Size distribution of glucose−PEG−PLA(54−48) micelle determined by DLS (histogram analysis).

Table 4. Size and Polydispersity Index of Glucose−PEG−PLA and Galactose−PEG−PLA Micelles

polymer	cumulant diameter (nm)	polydispersity index	weight ratio of PLA to PEG
glucose−PEG−PLA(54−48)	42.2	0.073	0.89
galactose−PEG−PLA(61−22)	37.6	0.090	0.39

Galactose- as well as glucose-bearing polymer micelles have a narrow distribution with a PDI lower than 0.1, indicating that the sugar-bearing polymer micelles have a narrow size distribution in aqueous medium. The diameter of the polymer micelles was then calculated from the diffusion coefficient determined at 90° based on eq 4 (Stokes−Einstein equation), and the results for both glucose−PEG−PLA and galactose−PEG−PLA micelles are summarized in Table 4 together with the values of PDI.

The γ-, number-, and weight-averaged distribution, respectively, of the glucose−PEG−PLA micelle, determined by the histogram analysis, are shown in Figure 8. Apparently, the polymer micelle had a unimodal size distribution with a gamma-averaged diameter of ∼40 nm. This diameter of the micelle is consistent with the core−shell architecture in which the end-to-end distance of the shell-forming PEG segment is assumed to be in the range calculated from random and meander models.[64]

The narrowly distributed nature as well as the appropriate size range (∼40 nm) to ensure tissue penetration lends the polymer micelle from sugar−PEG−PLA promising feasibility as a carrier for drug targeting. It should be noted that the polymer micelle prepared here has a size range comparable to previous polymer micelle systems showing good extravasation properties into solid tumors.[22,23]

Interaction between Sugar-Bearing Polymer Micelles and RCA-1 Lectin. To analyze the binding ability of the sugar-bearing PEG−PLA micelle toward lectin, the polymer micelle loaded with a fluorescent probe, pyrene, was passed through a column packed with RCA-1 immobilized beads. RCA-1 is one of the well-studied plant lectins which specifically recognize a β-D-galactose residue.

As seen in Figure 9, the MeO− and glucose−PEG−PLA micelles eluted at an exclusion volume (1.2 mL) as a sharp peak in the chromatogram, indicating no interaction of these polymer micelles with immobilized RCA-1 lectin. On the other hand, the major fraction of the galactose−PEG−PLA micelle was obviously retained in the column, indicating a strong interaction with RCA-1 lectin. This result confirms the existence of the galactose residues on the outermost region of

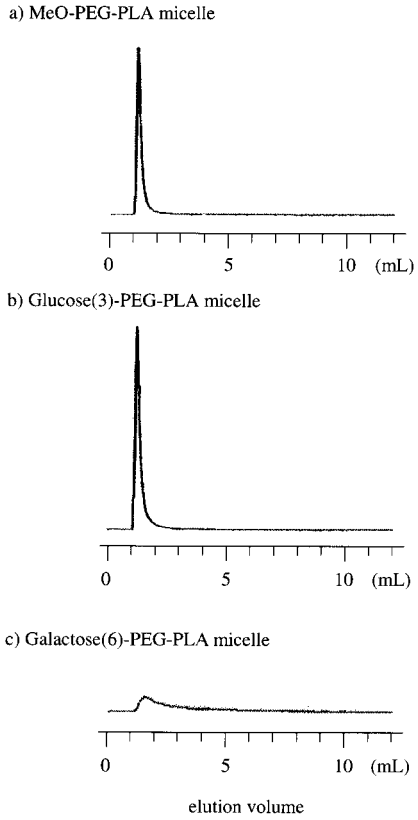


Figure 9. Elution profile of polymer micelles from the RCA-1 immobilized column. (Column volume, 8ϕ × 50 mm; injection volume, 4 μL; flow rate, 0.8 mL/min; temperature, 40 °C; detection, fluorescence (E_x = 339 nm, E_m = 375 nm).)

galactose−PEG−PLA micelle with a high availability to the sugar-binding site of the RCA-1 lectin molecule. This integrated expression of sugar groups on the micelle exterior is an advantageous characteristic of amplify their binding to a cellular glyco-receptor through multipoint recognition as is the case observed with several types of sugar-bearing polymers,[39−43] lending further ability for glyco-receptor-directed targeting to polymer micelle systems.

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