Articles

Vinyl Sulfone-Terminated PEG−PLLA Diblock Copolymer for Thiol-Reactive Polymeric Micelle

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ABSTRACT: Vinyl sulfone-terminated polyethylene glycol-poly(L-lactide) (VS−PEG−PLLA) diblock copolymer was designed as an amphiphilic structure containing a VS group with high reactivity toward thiols. Prior to the copolymer synthesis, heterobifunctional PEG with a VS group at one terminus was prepared and then PLLA was introduced to the other end of PEG. The chemical structure and molecular weight of the resulting products was assigned by ^{1}H NMR and GPC. As micellar properties of VS−PEG−PLLA under aqueous environment, the critical micelle concentration and mean size of VS−PEG−PLLA were found to be approximately 59.8 mg/L and 34 nm, respectively. Kinetic studies of the VS-functionalized PEG−PLLA micelle showed that the reaction rates were affected by various factors but most of cysteine-contained peptides reacted rapidly within 1 h *via* Michael-type addition. Therefore, this thiol-reactive polymeric micelle is expected to be very useful for a functional nanocarrier system through the effective conjugation of cysteine-containing peptides or proteins.

Introduction

With the significant progress in nanotechnology observed over the past 2 decades, there has been increasing demand for the development of targeted nanocarrier systems in the field of cancer nanotechnology related to drug delivery and imaging.[1−3] Accordingly, recent studies of cancer-specific chemotherapy have focused on the design of targeted nanocarriers through functionalization with the targeting molecules on the surface of various nanocarriers such as liposomes, micelles, polyplexes, and dendrimers.[3−5] These functionalized nanocarriers can also accumulate effectively around cancerous tissues in a size-dependent manner, i.e., the enhanced permeation and retention (EPR) effect.

Functionalized polymeric micelles are rapidly becoming powerful nanomedicine platforms in the area of cancer treatment on account of their many advantages.[6] Many types of block copolymers have been used for micelle formation *via* self-assembly, but continuous requirements of the copolymers with both biocompatibility and biodegradability have limited the choice of materials as a nanocarrier system for cancer treatment. For the hydrophilic component, poly(ethylene glycol) (PEG) is the most commonly used polymer owing to its excellent properties (e.g., non-toxicity and enhanced circulation time) for pharmaceutical applications.[6] In the case of a hydrophobic core that can be varied according to the purpose of the study, aliphatic polyester−PEG micelles such as PLA−PEG, PLGA−PEG, and PCL−PEG, have attracted considerable attention owing to their high biocompatibility and biodegradability.[7,8] However, in terms of active targeting of these micelles to cancer, PEG needs to retain different functionalities because aliphatic polyester−PEG copolymers can be prepared by ring-opening polymerization, which is polymerized from the hydroxyl groups of PEG. Several types of heterobifunctional PEG are now commercially available for the design of functionalized polymeric micelles. In some cases, they can be modified to obtain the desired PEG functionality.[9−11] To date, many studies have examined functionalized aliphatic polyester−PEG micelles using hetrobifunctional PEG.[12−15] However, the applications of these reactive block copolymers or micelles have some limitations such as harsh conditions, low conjugation yield, complicated processing, and slow reaction rates for the extensive use of a variety of cancer targeting molecules.

The Michael-type addition, which commonly refers to the conjugation reaction between activated electrophilic olefin and nucleophiles, has been extensively applied in biomedical fields because it occurs rapidly without side products under mild reaction conditions.[16] In particular, the reaction is quite effective in generating polymer-based bioconjugates with cysteine-terminated peptides or proteins, and it can be accomplished mainly using PEG derivatives modified with vinyl sulfone or maleimide as the preferred end groups.[17] Although PEG−maleimide is widely used to make targeted and long-circulating nanocarriers,[18−20] it is reported that PEG−vinyl sulfone has several advantages over PEG−maleimide with a relative fast reaction rate. The specificity is slightly better at pH below 8 and the thioether linkage formed with thiol compounds is a great deal more stable than the linkage formed with maleimides.[21] In addition, the VS group is quite stable in aqueous solution.[22,23] On the basis of these advantages, Rehor et al. have recently reported surface functionalization of poly(propylene sulfide) (PPS) nanoparticles using Pluronic−vinyl sulfone modified with divinyl sulfone.[24]

This study deals with micellar properties of VS-terminated PEG−PLLA diblock copolymer in aqueous media, including polymer synthesis and characterizations. To allow thiol-reactivity of the copolymer, heterobifunctional PEG with a VS group at one terminus was prepared by a multi-step synthetic procedure

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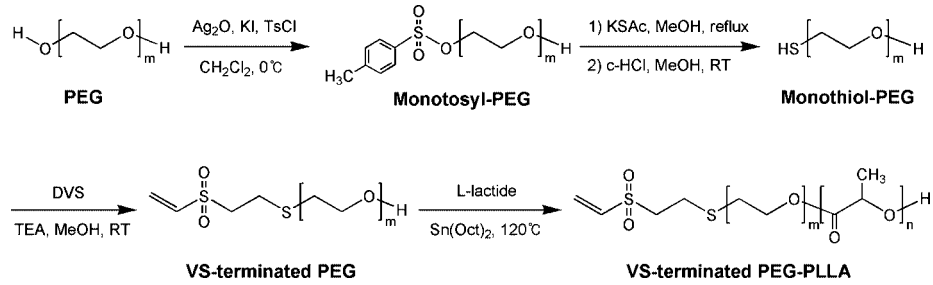

Figure 1. Synthetic routes to VS-terminated PEG−PLLA diblock copolymer.

and PLLA was then introduced to the remaining terminal hydroxyl group of PEG using a conventional method. Micellar properties of the obtained block copolymer were characterized, and the reactivity of the VS-functionalized micelle toward thiols was investigated using peptide-based cancer targeting molecules.

Experiments

Materials and Methods. Poly(ethylene glycol) (PEG, M_w 3400) with a pharmaceutical grade was purchased from Polysciences (Warrington, PA). Potassium iodide, silver (I) oxide, *p*-toluenesulfonyl chloride (TsCl), potassium thioacetate (KSAc), and stannous 2-ethylhexanoate were obtained from Sigma Aldrich (St. Louis, MO). Divinyl sulfone (DVS) and L-lactide were supplied by TCI (Tokyo, Japan) and Boehringer-Ingelheim (Ingelheim, Germany), respectively. Cys-Arg-Glu-Lys-Ala (CREKA, purity >95%) and TAT (CKYGRRRQRRKKRG, purity >95%) were supplied by Peptron (Daejeon, Korea). Cyclo(Arg-Gly-Asp-D-Phe-Cys) (c(RGDfC)) was purchased from Peptide International (Louisville, KY). All reagents and solvents were used as received without further purification.

Synthesis of Monotosyl−PEG. Monotosyl−PEG was synthesized using a stoichiometric amount of *p*-toluenesulfonyl chloride in the presence of silver(I) oxide and a catalytic amount of potassium iodide, as previously reported.[25] First, 10 g of PEG was dissolved in 200 mL of methylene chloride and maintained at 0 °C. Freshly prepared silver(I) oxide (1.02 g, 4.41 mmol) and potassium iodide (196 mg, 1.18 mmol) were then added to the rapidly stirred PEG solution. The reaction after adding TsCl (0.59 g, 3.09 mmol) was allowed to proceed for 3 h at 0 °C. The mixture was filtered over a Celite pad and evaporated completely. Then, the precipitates, followed by addition of methanol, were removed by membrane filtration and which were evaporated. The resulting crude powders were dissolved in methylene chloride, precipitated in cold diethyl ether, and dried *in vacuo* to give a white powder. The yield and degree of substitution were approximately 90% and 85%, respectively, as calculated from the integral values of PEO (δ 3.9−3.4) and the methylene protons adjacent to the tosyl group (δ 4.15). ^{1}H NMR ($CDCl_3$, δ in ppm): 7.79 (d, Ar), 7.34 (d, Ar), 4.15 (t, CH_2OTs), 3.63 (m, O−(CH_2)$_2$−O), 2.84 (s, CH_2−OH), 2.63 (s, O*H*), and 2.45 (s, Ar−CH_3).

Synthesis of Monothiol−PEG and VS Conjugation. The obtained monotosyl−PEG (8 g, 2.25 mmol) and potassium thioacetate (2.56 g, 22.4 mmol) were dissolved in 200 mL of methanol, and the mixture was heated under reflux for 3 days under nitrogen atmosphere. The solution was allowed to cool to room temperature, and then evaporated. The crude solid was dissolved in a small volume of methylene chloride, filtered and evaporated. Then 10 mL of concentrated HCl was added to 100 mL of a methanolic solution of monothioacetate−PEG, and the mixture was stirred for 24 h at room temperature. After the reaction, the methanol was evaporated completely before adding an excess volume of methylene chloride. The residual water was then dried over $MgSO_4$. Concentration under reduced pressure afforded the crude product, which was precipitated with a cold diethyl ether and dried *in vacuo*. ^{1}H NMR ($CDCl_3$, δ in ppm): 3.63 (m, O−(CH_2)$_2$−O), 2.91 (s, CH_2−OH and CH_2−SH).

As a final step of PEG modification, the conjugation of DVS to monothiol−PEG was carried out using the following procedure. Monothiol−PEG (α-hydroxyl-ω-mercapto PEG) was dissolved in 100 mL of methanol containing triethylamine (TEA, 300 mg, 2.97 mmol). An excess amount of DVS (20-fold) was added quickly to the mixture and reacted for 2 h at 40 °C. After evaporation, the concentrated solution was precipitated with cold diethyl ether and dried *in vacuo*. The product was stored at a dry keeper prior to use. ^{1}H NMR ($CDCl_3$, δ in ppm): 6.8 (dd, SO_2CH), 6.4 (d, CH=CH_2), 6.1 (d, CH=CH_2), 3.63 (m, O−(CH_2)$_2$−O, SO_2CH_2), 2.65 (m, CH_2−S, CH_2−O).

Synthesis of VS-Terminated PEG−PLLA Diblock Copolymer. The VS-terminated PEG−PLLA diblock copolymer (VS−PEG−PLLA) was synthesized by ring-opening polymerization of L-lactide using VS-terminated PEG as an initiator. L-Lactide (0.743 g, 5.16 mmol) and VS-terminated PEG (3 g, 0.86 mmol) were placed in a glass ampule, containing 0.06 g of stannous 2-ethylhexanoate as a catalyst. The ampule was evacuated and then sealed. The sealed ampule was immersed in a mineral oil bath and reacted for 6 h at 120 °C. After polymerization, the resulting product was dissolved in methylene chloride and precipitated with a cold diethyl ether. ^{1}H NMR ($CDCl_3$, δ in ppm): 6.8 (dd, SO_2CH), 6.4 (d, CH=CH_2), 6.1 (d, CH=CH_2), 5.20 (m, OOC−CH(CH_3)), 4.25 (b, CH_2OOC) 3.63 (m, O−(CH_2)$_2$−O), 2.91 (m, CH_2−OH and CH_2−SH). 1.54 (m, OOC−CH(CH_3)). Figure 1 shows the total synthetic routes to the VS-terminated PEG−PLLA.

Polymer Characterizations. The chemical structures of the polymers corresponding to each synthetic procedure were analyzed by ^{1}H NMR (Mercury 400 MHz, Varian Co., USA). The molecular weight and molecular weight distribution of VS-terminated PEG−PLLA were determined using gel permeation chromatography (GPC) apparatus equipped with 515 HPLC pump, 717 plus auto sampler and 419 differential refractometer (Waters Co., USA). Tetrahydrofuran was used as the solvent, and polystyrene standards (Polysciences Inc., Warrington, PA) were used to generate a GPC calibration curve. The molecular weight was also estimated by integrating the methylene protons in the PLLA and PEG segments.

Preparation and Characterizations of VS-Functionalized PEG−PLLA Micelles. A 200 mg sample of the copolymer was dissolved in 40 mL of dimethyl sulfoxide. The polymer solution was transferred to a pre-swollen membrane (Cellu•Sep, molecular weight cutoff size, 3500), dialyzed against water for 24 h and subsequently lyophilized. The yield of VS-functionalized PEG−PLLA micelle formation was approximately 80%.

The critical micelle concentration (cmc) of the copolymer was estimated by fluorescence spectroscopy using pyrene, a hydrophobic florescence probe, which was partitioned preferably in the micelle core, causing changes in the photophysical properties of the nanoparticles under investigation.[26] A saturated aqueous solution of pyrene (6×10^{-7} mol/L) was used for the measurements. The polymer concentration was varied from 1×10^{-4} to 1.0 g/L. The fluorescence excitation spectra were obtained with an emission wavelength of 390 nm as a function of the polymer concentration using spectrofluorometer (JASCO FP-6500, Japan). The size and size distribution of the micelles were measured by dynamic light scattering (Zetasizer 3000HS, Malvern, UK). The micellar morphology was analyzed by high resolution transmission electron microscopy (JEOL 300 kV, JEOL, USA). A drop of a 1 mg/ml micelle

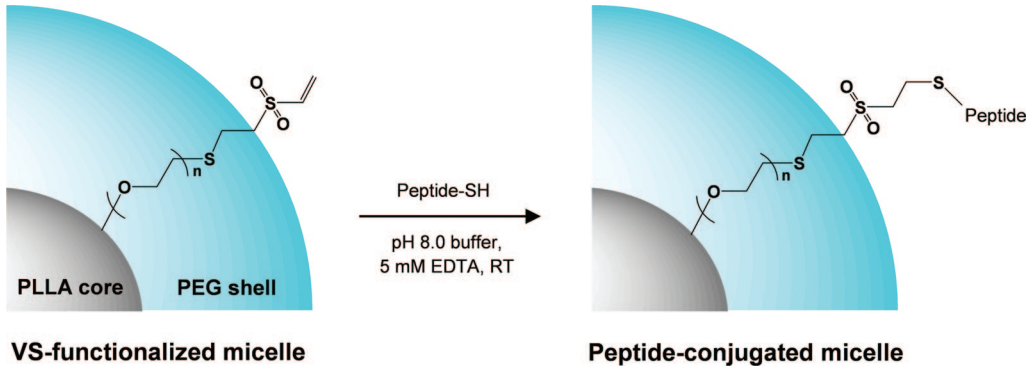

Figure 2. Michael type addition of cysteine-contained peptide to VS-functionalized PEG−PLLA micelle.

solution was placed on a 300 mesh copper grid coated with carbon. The sample was stained with 1% (w/v) phosphotungstic acid and dried at room temperature for 24 h.

Kinetic Studies of VS-Functionalized PEG−PLLA Micelles toward Thiols. The reactivity of the micelles toward thiols, cysteine-contained peptides, was evaluated by monitoring the thiol concentration using Ellman's method.[21] Four different thiols were used, L-cysteine, CREKA, c(RGDfC), and TAT. L-Cysteine was used as a control. To measure the reactivity of the VS-functionalized micelles with thiols, 4 mM of the micelles were dispersed in 1 mL of 0.1 M sodium phosphate buffer solution containing 5 mM EDTA (pH 8.0) and 2 mM of thiol was then added. At scheduled times, aliquots (30 μL) were withdrawn and quenched by addition to 920 μL of pH 7.27 buffer (0.1 M sodium phosphate, 5 mM EDTA). 50 μL of 5,5′-dithio-*bis*-(2-nitrobenzoic acid) (DTNB) was added. After 5 min, the absorbance at 412 nm was measured using UV-spectrophotometer (JASCO V-570, Japan). Michael type addition of cysteine-contained peptide on the surface of the VS-functionalized micelles is described in Figure 2.

Results and Discussion

Synthesis of VS-Terminated PEG and PEG−PLLA Copolymer. Heterobifunctional PEG with a VS group on one terminus and a hydroxyl group on the other was prepared using a multi-step synthetic procedure. Selective monotosylation of PEG diols is considered the most critical part of all reaction steps because the selective reaction can lead to a high yield of heterobifunctional PEG. Bouzide et al. reported the synthetic method to monotosylate symmetrical diols.[27] Although they used oligo(ethylene glycol)s (OEGs) ($<M_w$ 300) for monotosylation, Li and co-workers found that a considerable amount of monotoyl−PEG ($M_w = 1.5$K) could be obtained by controlling the amounts for feed each reagent.[10,25] On the basis of their results, the monotosylation of PEG with a relatively high molecular weight ($M_w = 3.4$K) was carried out because the chain length of PEG was not likely to highly associate with its selectivity. As shown in Figure 3a, the ^{1}H NMR spectrum of monotosyl−PEG shows the characteristic peaks for a tosyl group. The conversion yield was determined to be 85%, which was verified through the monotosylation of PEG more than 10 times.[28]

Monothiol−PEG was obtained through the following two steps: (1) the preparation of monothioacete−PEG; (2) the cleavage of *S*-acetyl groups from monothioacetate-PEG. In the latter case, the procedure was carried out without further purification of the monothioacetate−PEG because the hydrolysis step, followed by the addition of an excess of concentrated HCl, allowed ease of purification including the removal of potassium salts. Also, this acidic condition is preferred to protect side reactions compared to other conditions (i.e., Na/NH_3, $NaBH_4$, MeONa/MeOH).[29] Figure 3b and 3c shows the characteristic peaks of monothioacetate−PEG and monothiol−PEG. DVS was

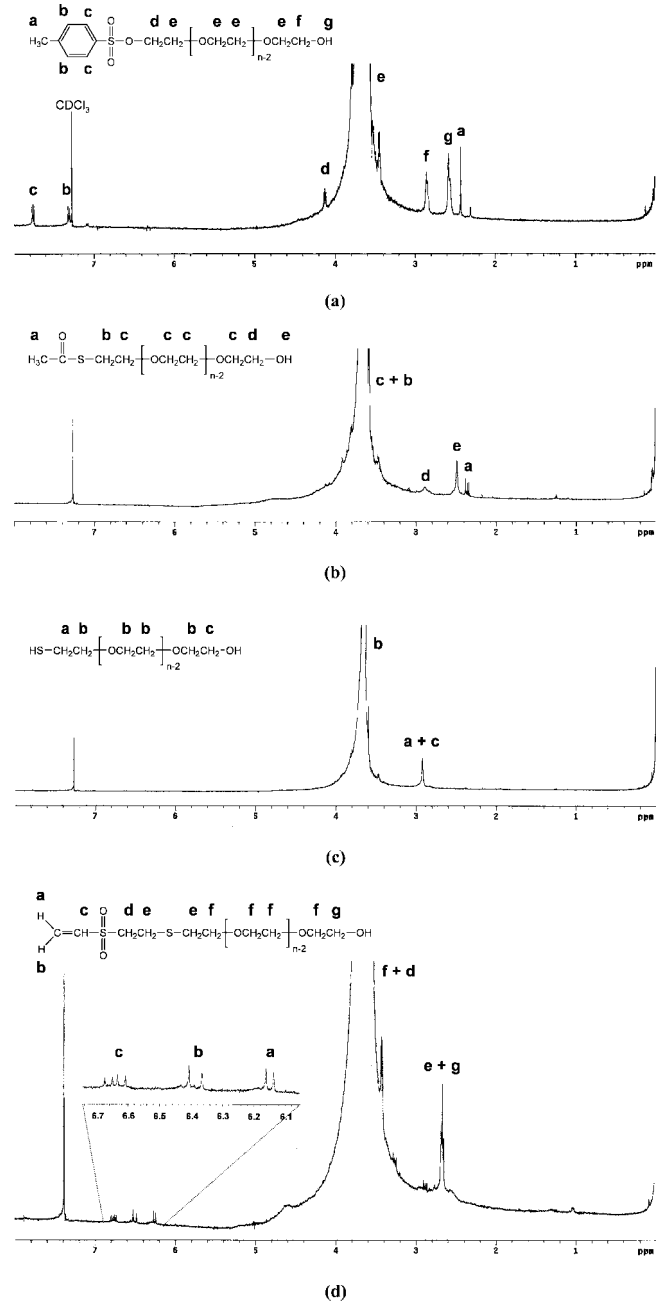

Figure 3. ^{1}H NMR spectra of monotosyl−PEG (a), monothioacetate−PEG (b), monothiol−PEG (c), VS-terminated PEG (d).

conjugated to monothiol−PEG. Figure 3d shows three distinct peaks at δ 6.8, 6.4, and 6.1 corresponding to the vinyl group of

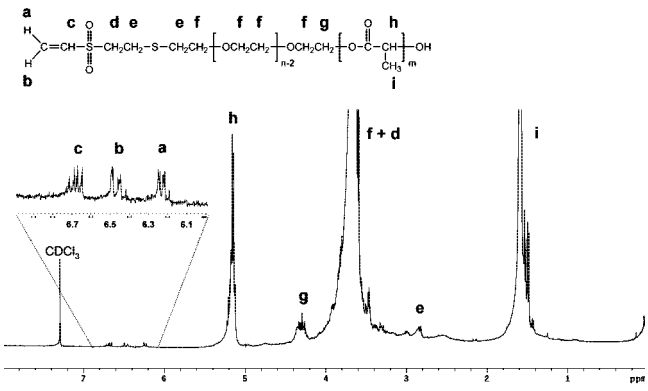

Figure 4. ^{1}H NMR spectra of VS-terminated PEG−PLLA diblock copolymer.

Table 1. GPC results of VS-Terminated PEG−PLLA Diblock and PLLA−PEG−PLLA triblock copolymers

			PLLA		
polymers	M_n[a]	M_n[b]	unit	M_n[b]	M_w/M_n[b]
VS−PEG−PLLA 1	6420	7360	55	3960	1.34
VS−PEG−PLLA 2	5420	6450	42	3050	1.24
PLLA−PEG−PLLA	4440	5660	31	2260	1.20

[a] Number-averaged molecular weight, M_n, estimated by ^{1}H NMR measurements in $CDCl_3$. [b] Determined by GPC analysis.

VS-terminated heterobifunctional PEG. In addition, it was confirmed that the hydroxyl groups of PEG showed no reactivity with DVS under mild basic conditions. As previously described in several papers, VS-terminated PEG could be obtained by reacting hydroxyl groups of PEG with an excess amount of DVS. On the other hand, strong bases such as sodium hydride and potassium *tert*-butoxide were required to increase the nucleophilicity of the hydroxyl groups.[30,31] Accordingly, the reactivity of unmodified PEG against DVS is strictly limited under our synthetic conditions.

VS-terminated PEG−PLLA, amphiphilic biodegradable block copolymer with a thiol-reactive functionality, was prepared by the conventional polymerization of L-lactide using VS-terminated PEG as a macroinitiator. The resulting structure in Figure 4 shows two peaks for the PLLA units as well as the retained VS functionality. The results of GPC analysis and ^{1}H NMR estimation of the molecular weight of copolymers are summarized in Table 1. Varying the feed ratio of L-lactide, the PLLA block length could be well-controlled with a relatively narrow distribution. In contrast, PLLA−PEG−PLLA triblock copolymer prepared from the unmodified PEG showed a significant difference in the number of PLLA repeating units compared to the VS-terminated PEG−PLLA. These results support that VS-terminated PEG−PLLA was prepared successfully from VS-terminated PEG.

Cmc and Size Distribution of VS-Functionalized Micelles. Since aliphatic polyester-based micelles, such as PEG−PLA, PEG−PLGA, and PEG−PCL, have been exploited, there has been considerable effort to identify the significance of a cmc value as well as cmc-related factors. The cmc is considered an indicator of the physical properties and the stability of the micelles under aqueous environment, which is known to be strongly affected by both the PEG block length and the hydrophobic content.[32,33] As shown in Figure 5, plotting the ratio of the intensity of the signal at 339 nm to that of the signal at 334 nm versus the logarithm of the VS-terminated PEG−PLLA (34/30) concentration by fluorescence spectroscopy using pyrene resulted in a cmc of 59.8 mg/L. This value is relatively high compared to previous results that exhibited cmc values ranging

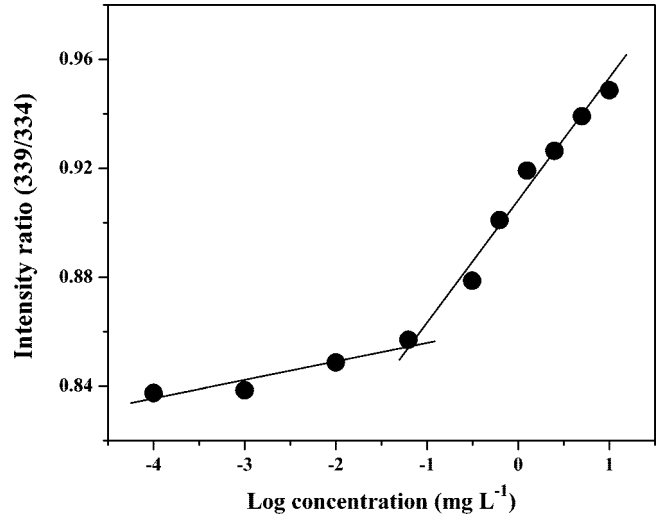

Figure 5. Intensity ratio of pyrene as a function of VS-terminated PEG−PLLA (34/30) concentration.

from 2 to 5 mg/L according to various PLA block lengths.[34] A high cmc explains that the copolymer has a longer PEG segment relative to a certain PLA length (i.e., 350 mg/L for PEG/PDLLA (18/9)), simultaneously indicating that the micelles are relatively unstable compared to the copolymer with a low cmc.[33] Dai et al. demonstrated that the role of the PLA block is more crucial than that of the PEG length in micelle formation when the PLA block chain is long enough.[35] In addition, Yasugi et al. reported that the polydispersity of block copolymers can significantly influence the formation of micelles.[36] As VS-terminated PEG−PLLA (34/30) has a little longer PEG length than PLLA, the difference between the two blocks is less likely to affect the cmc. On the contrary, it is believed that a high cmc value seems to be derived from the polydispersity of the constituent block copolymer (PDI, 1.24) as a result of the presence of triblock copolymers obtained from a small fraction of unmodified PEG. This is consistent with previous results showing that block copolymers with relatively short blocks of PLLA lead to a high cmc.[37,38] The size distribution and shape of the VS-functionalized PEG−PLLA (34/30) micelles are presented in Figure 6. The average size of the micelles was approximately 33.5 ± 3.0 nm with a polydispersity of 0.242. A TEM image indicates the micelles to be spherical in shape, which is almost similar to the result obtained by DLS analysis.

Kinetic Studies of VS-Functionalized Micelles with Thiols. This study investigated the reaction rates of the VS-functionalized micelles with thiols, such as CREKA, c(RGDfC), and TAT, which are widely used as cancer targeting ligands.[19,39,40] Michael-type addition is affected by many factors but especially, the solution pH plays a critical role in controlling the reaction rate.[21] In order to investigate the reaction rates of each thiol at the same pH, all thiol compounds were dissolved in a pH 8 buffer solution and reacted with VS-functionalized micelles. Figure 7 shows a time-dependent disappearance of free thiols in each mixture. Adding thiols to micelle solutions, all thiols began to quickly consume within 1 h while the rate of decrease differed according to thiols. Both L-cysteine and CREKA showed similar patterns until 4 h, indicating that their reaction rates were almost the same at pH 8. As described in Table 2, however, it was found that the rate constants of L-cysteine were slightly larger than those of CREKA and it had the shortest half-life among thiols. TAT reacted rapidly with the micelles, while the disappearance of free TAT was reduced after 1 h. Interestingly, c(RGDfC) had the slowest reaction rate, resulting from the limited solubility of c(RGDfC) in aqueous

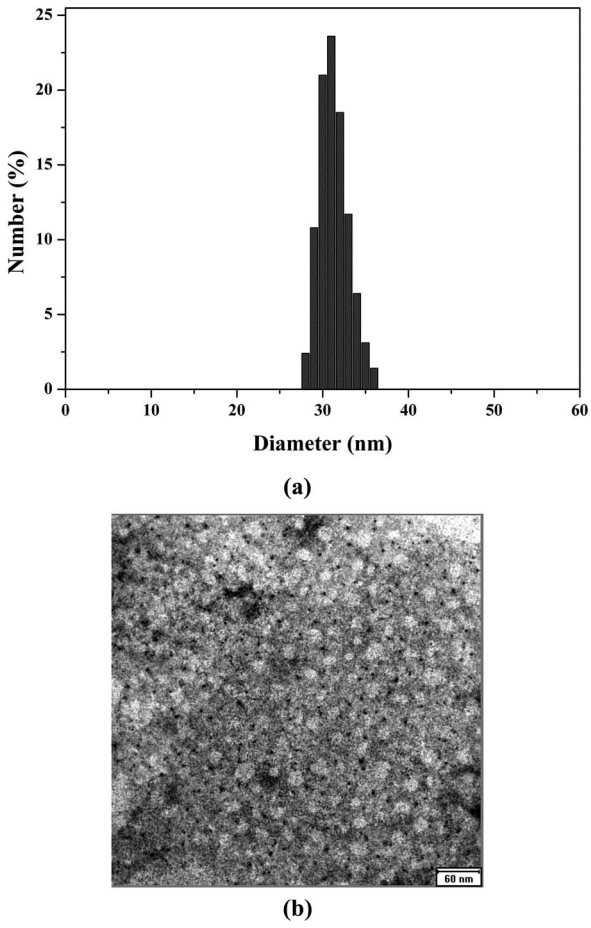

Figure 6. Size distribution (a) and TEM image (b) of VS-functionalized PEG−PLLA micelles.

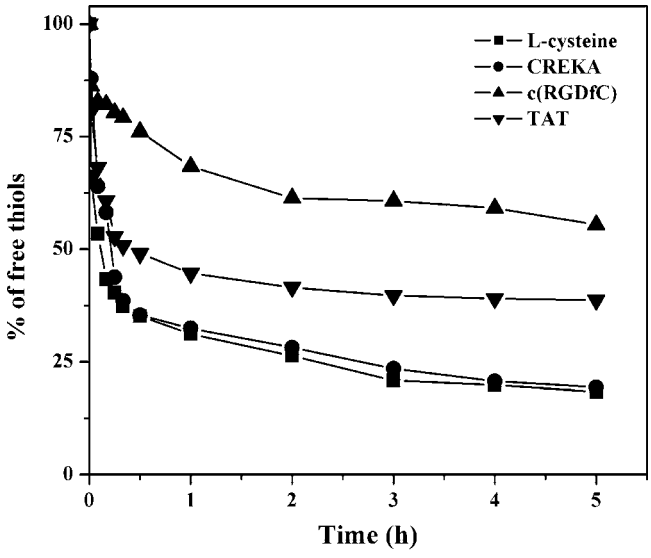

Figure 7. Reaction between VS-functionalized PEG−PLLA micelles and thiols as evaluated from the disappearance of free cysteines. The reaction was carried out at room temperature. [VS−PEG−PLLA] = 4 mM; [SH] = 2 mM.

media. On the basis of the protocol for manufacture, 1 mg of c(RGDfC) was dissolved in 100 μL of 3% (v/v) acetic acid and then diluted with 900 μL of a pH 8 buffer solution. After mixing with 1 mL of a pH 8.0 buffer solution, the solution pH changed from 6.1 to 7.0. Therefore, the results of c(RGDfC) obtained at low pH were not comparable with those of other peptides. However, it is concluded that the reaction of c(RGDfC) with micelles is strongly affected by a relatively low pH compared with the other reaction buffers at pH 8.0, indicating that a solution pH is one of critical experimental parameters for the Michael-type reaction. The results for both the rate constants and half-life also suggest that c(RGDfC) has the slowest reaction rate.

Table 2. Approximate Rate Constants for the Reaction of VS-Functionalized PEG−PLLA Micelles with Thiols

thiols	k_2 (M^{-1} min^{-1})[a]	k_1 (min^{-1})[b]	$t_{1/2}$ (min)[c]
L-cysteine	14.18	0.057	12.2
CREKA	13.35	0.053	13.0
c(RGDfC)	1.70	0.007	101.9
TAT	10.98	0.044	15.8

[a] The second-order rate constant (k_2) was obtained by dividing the slope of the ln A versus time plot by the concentration of the copolymer. [b] The first-order rate constant (k_1) is the slope of the ln A versus time plot. [c] The half-life ($t_{1/2}$) was obtained by dividing the ln2 by k_1.

The concentration of all free thiols decreased gradually, even after 4 h. However, it should be noted that the remaining thiols attained equilibrium-like states, indicating that a small amount of thiols remains unreacted. As described in the experimental section, kinetic studies were carried out in a small volume of a mixture which contains a highly concentrated micellar solution (>200-fold of cmc). However, the above conditions can be an obstacle for a complete reaction. There are several physical and biochemical parameters for surface reactions, as recently reported by Gindy et al.[41] In the present case, the simultaneous alignment of thiols at the reactive micellar surfaces appeared difficult due to steric hindrance by the covalently bound thiols. This aspect can be supported by the exponential decrease in the amount of free thiols at the initial step, representing abundant VS functionality. In addition to the poor access of thiols, the unfavorable diffusion of thiols along the packed micelles in the mixture may influence the reaction. On the basis of the reaction chemistry, the Michael-type reactivity of thiols also needs to be considered for a better understanding. Lutolf et al. demonstrated that the peptide primary structure has significant influence on thiol reactivity. Consequently, positively charged amino acids close to the SH groups can increase the reaction rate by decreasing the pK_a of the thiol.[42] As CREKA has an arginine residue close to cysteine, it is expected that this sequence will increase the reaction rate of CREKA, even though the molecular weight of CREKA is larger than that of L-cysteine. TAT possesses a positively charged lysine residue close to cysteine but the poor access of these peptides with a high molecular weight is more likely to contribute to its reaction rate.

This study focused on the development of a new functional copolymer for the preparation of reactive polymeric micelles. Based on its thiol-reactive functionality on the micellar surface, the relative reaction rates of thiols were examined with four types of cysteine-contained peptides under aqueous conditions. Since surface presentation of targeting ligands *via* postassembly is particularly beneficial for intracellular uptake of micelles,[6] the VS-terminated PEG−PLLA copolymer has a great advantage for the rapid conjugation of cysteine-contained peptides containing a cysteine residue. In order to optimize this approach, various parameters are needed to consider the surface density of targeting ligands. In case of drug encapsulation, however, preassembly approach is more effective in preventing the loss and initial release of encapsulated drugs that can occur during ligand conjugation. Alternatively, the conjugation of cysteine-contained peptides can be achieved with DMSO, which dissolves both VS-terminated copolymer and peptide. It is also possible to incorporate drugs into peptide-conjugated micelles during micelle formation.

Conclusions

In this study, VS-terminated PEG−PLLA has been developed as an amphiphilic diblock copolymer with high reactivity toward thiols. Based on effective PEGylation chemistry of proteins or peptides *via* Michael-type addition, one terminus of heterobifunctional PEG was designed to possess a VS group which is advantageous for reaction rates, yields, and stability. Consequently, PLLA was introduced to the VS-terminated PEG. The resulting copolymer was characterized successfully and its general features in the micellar state were examined. Especially, the most attractive feature of VS-terminated PEG−PLLA is to facilitate surface functionalization of the micelles with a wide range of peptide-based ligands which contain a cysteine residue. Since cancer treatment has been very complicated according to cancer patients, the case-by-case studies using nanocarriers are essential for patient-customized treatments. Therefore, it is expected that the convenient introduction of cancer targeting ligands to VS-functionalized micelles will provide further opportunities for extensive studies of the drug-encapsulated micelles against various types of cancer.

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