Core-Polymerized Reactive Micelles from Heterotelechelic Amphiphilic Block Copolymers

Michihiro Iijima,† Yukio Nagasaki,*,† Takashi Okada,† Masao Kato,† and Kazunori Kataoka*,‡

Department of Materials Science and Technology, Science University of Tokyo, Yamazaki 2641, Noda, Chiba 278-8510, Japan, and Department of Materials Science, Graduate School of Engineering, The University, of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8656, Japan

Received October 13, 1998; Revised Manuscript Received December 1, 1998

ABSTRACT: Amphiphilic poly(ethylene glycol)-*b*-polylactide (PEG/PLA) copolymers with an aldehyde group at one end and a methacryloyl group at the other chain end were synthesized by anionic polymerization. The efficiencies of the functionalization at both ends were almost quantitative. The amphiphilic block copolymers formed micelles in aqueous media. Acetal groups on the micelle surface were quantitatively converted to aldehyde groups by an acid treatment. The end methacryloyl group located in the core of the micelle was polymerized effectively to form core−shell-type nanoparticles having reactive aldehyde groups on the surface. The size of the reactive nanoparticle was 20−30 nm which was constant with temperatures up to 60 °C. The stability of the micelle was also confirmed by a sodium dodecyl sulfate (SDS) treatment. When SDS was added to the nanosphere solution to 20 mg/mL, the particle was not collapsed. The particle was stable enough even in organic solvents. This functionalized micelle having high stability is not only expected to have wide utilities in biomedical applications (including drug delivery, diagnosis, and surface modification through the coupling of bioactive substances) but also to be of great interest as a novel supramolecular architecture.

Introduction

A polymeric particle with micro- to nanometer diameter is attractive in the field of nanofabrication chemistry.[1] Polymeric particles of nanodimension in diameter are especially important as novel drug delivery systems in biomedical applications. A dendrimer is well-known to provide particles of a few nanometers in diameter.[2] In general, a dendrimer is prepared by successive 1:2 consecutive reactions to form a dendritic structure. Thus, the surface of the dendrimer possesses many reactive groups. This is one of the reasons for the utilization of the dendrimer as the starting material in nanofabrication chemistry. However, it is difficult to complete the consecutive reactions for the preparation of dendrimers. Actually, defects often appear after more than the four generations. These defects sometimes induce a serious problem for nano-supramolecular fabrication and also for the applications of the supramolecules such as in a drug delivery system.

Amphiphilic AB block copolymers form micellar structures in selective solvents.[3] Though these nanospheric particles are formed by intermolecular interactions of one of the block segments, which is insoluble in the selective solvents, they are fairly stable compared with low molecular weight micelles. Such polymeric micelles tend to form a spherical structure of a few tens to a few hundreds of nanometers in diameter. The size of the nanoparticle was promising not only as a drug targeting carrier but also in nanofabrication chemistry. However, most of the polymeric micelles prepared so far possess no reactive group on the surface.[4]

Recently, we reported a facile and quantitative synthetic method for the formation of the heterobifunctional poly(ethylene glycol),[5−9] which denotes PEG having a functional group at one end and another functional group at the other end. When one of the functional end groups in the heterobifunctional PEG selectively initiates the polymerization of a hydrophobic monomer, a new heterobifunctional AB block copolymer can be created, retaining the other functional group at the PEG chain end. In our previous work,[10,11] lactide was chosen as the hydrophobic segment, because (i) the ring-opening polymerization of lactide can be initiated by potassium alcoholate at the living PEG chain end without any side reaction, (ii) PLAs are biodegradable and nontoxic and are widely utilized as implant materials, and (iii) nanoparticles consisting of block copolymers of α-methoxypoly(ethylene glycol) and PLA are suitable for drug delivery. The PEG/PLA block copolymer having a functional group at the PEG end provides a polymeric micelle possessing functional groups on the surface. These reactive micelles are promising for biomedical applications such as drug targeting.

For nanofabrication chemistry utilizing the surface reactive groups, however, the physical coagulation force of the hydrophobic core may not be stable enough. The objective of this work was to create stable nanospheres having reactive groups on the surface as a starting tool for nano-supramolecular fabrication (Figure 1). To stabilize the micelle, aldehyde-PEG/PLA-methacryloyl which has a polymerizable group at the PLA end was quantitatively synthesized and used for the creation of a stable nanosphere having aldehyde groups on the surface.

Experimental Section

Materials and Methods. Commercial tetrahydrofuran (THF), 3,3-diethoxypropanol (Aldrich), 2-methoxyethanol (WAKO), ethylene oxide (EO) (Sumitomo 3M), DL-lactide (LA) (Tokyo Kasei), and methacrylic anhydride (Aldrich) were

* Corresponding authors.
† Science University of Tokyo.
‡ The University of Tokyo.

10.1021/ma9815962 CCC: $18.00
Published on Web 01/27/1999

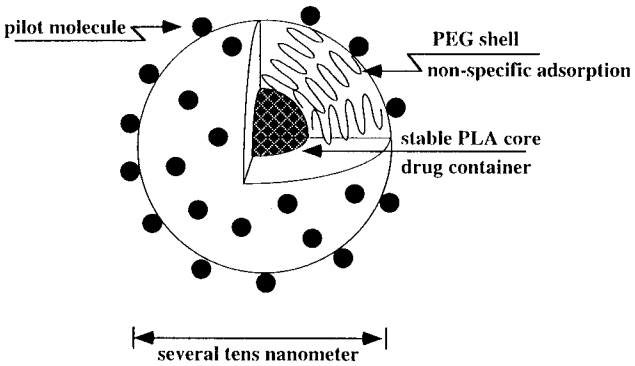


Figure 1. Fabrication of reactive nanosphere.

purified conventionally. Potassium naphthalene was used as a THF solution, whose concentration was determined by titration. Isopropyl alcohol and dimethyl acetamide were used as received.

Analysis. GPC measurements in organic solvents were carried out using a TOSO HLC-8020 equipped with a Shodex gel permeation column (Shodex KD-806M•2). DMF containing 10 mmol L^{-1} lithium bromide was used as the eluent at a flow rate of 1.0 mL min^{-1} at 40 °C. GPC measurements in water were carried out using a JASCO HPLC system equipped with a Shodex gel permeation column (Shodex GF-7MHQ) and an internal RI detector (RI-930). Water containing 0.1 wt % sodium azide was used as the eluent at a flow rate of 0.5 mL min^{-1} at 25 °C. ^{1}H NMR spectra were obtained using chloroform-*d* solutions (1.0 wt %) with a JEOL EX400 spectrometer at 400 MHz. Chemical shifts relative to $CHCl_3$ (1H: $\delta = 7.26$) were employed. A light-scattering spectrometer (DLS-7000 Photal, Otsuka Electronics) equipped with a 75 mW Ar laser that produces vertically polarized incident beams at $\lambda_0 = 488$ nm was used in the present study for dynamic and static light scattering measurements.

Polymer Synthesis. One of the representative procedures for the preparation of α-acetal-ω-methacryloyl-PEG/PLA block copolymer was described. α-Acetal-ω-methacryloyl-PEG/PLA block copolymers have been synthesized by a one-pot anionic ring-opening polymerization of EO followed by LA initiated with potassium 3,3-diethoxypropanolate (PDP) as an initiator at room temperature under argon. One millimole (0.16 mL) of 3,3-diethoxypropanol and 1 mmol of potassium naphthalene were added to 30 mL of dry THF to form PDP. After stirring for 10 min, 130 mmol (6.5 mL) of condensed EO was added via a cooled syringe to the formed PDP solution. The polymerization of the EO proceeded for 2 days at room temperature, resulting in highly viscous solution. Potassium naphthalene (about 0.1 mmol) was added until the solution turned pale green to stabilize the living chain end, 35 mmol (32.0 mL) of an LA solution in THF ($c = 1.10$ mol/L) was introduced, and the polymerization proceeded for 120 min. After the polymerization, 20 mmol (3.10 mL) of a methacrylic anhydride was introduced into the polymer solution, and the reaction proceeded for 2 days. The polymer was recovered by precipitation into a 20-fold excess of cold isopropyl alcohol (−15 °C), stored for 2 h in the freezer, and centrifuged for 30 min at 6000 rpm. The polymer was then freeze-dried with benzene. The yield of the obtained polymer was ca. 90%.

Polymer Characterization. The molecular weight of the PEG prepolymer was determined by GPC using DMF as the eluent at the end of the EO polymerization. PEG standard samples were utilized to calibrate the molecular weight. The molecular weight of the PLA segment was determined using an ^{1}H NMR spectrum by the ratio of methine protons in the PLA segment and methylene protons in PEG segment based on the M_n of PEG determined from the GPC results. The extent of the conversion of the acetal to aldehyde groups at the end of the polymer chain was estimated by ^{1}H NMR spectroscopy after acid treatment and purification.

Micelle Preparation. The procedure has been previously detailed.[10,11] Briefly, 280 mg of the copolymer was dissolved in 40 mL of dimethylacetamide, and the polymer solution was transferred into a preswollen membrane tube (Spectra/Por molecular weight cutoff size 12 000−14 000), dialyzed against 2 L of water for 24 h with exchanging water at 2, 5, and 8 h passage.

To convert the α-acetal-terminated micelle into a micelle with aldehyde groups at the end of the PEG chain, the polymeric micelle solution was adjusted to pH 2 with HCl. After stirring for 2 h, the mixture was neutralized with NaOH, and the solution was dialyzed against water to remove the salt. The procedure was the same as previously described. The aldehyde micelle thus obtained was directly analyzed by DLS. A part of the micelle was frozen in liquid nitrogen and lyophilized for several measurements, resulting in a yield of about 90%.

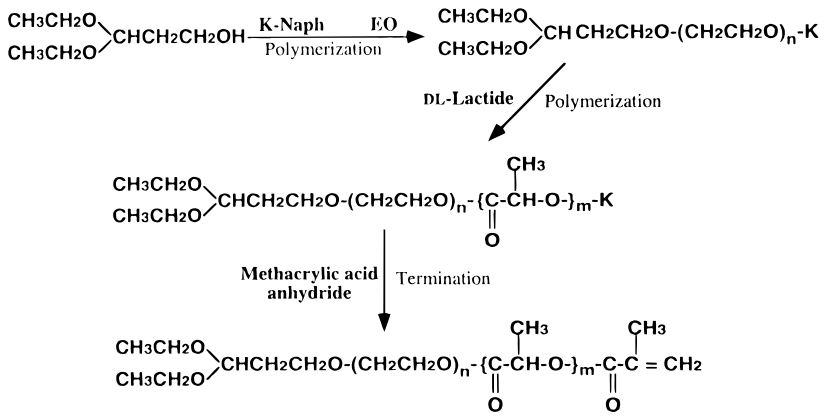
Polymerization of Methacryloyl Group in the Core of the Micelle. For core stabilization, the polymerization of the methacryloyl end group of the block copolymer in their aqueous micelle form was carried out. After azobis(2,4-dimethylvaleronitrile) (1.0 wt % polymer, V-65, Wako, $t_{1/2} = 51$ °C) solution in CH_2Cl_2 (1.5 mg/mL) was added into a micelle aqueous solution in a 300 mL flask, the mixture was allowed to stir for 2 h at room temperature in order to disperse the initiator to each of the micelle cores and evaporate the methylene chloride. The resulting micelle solution was purged with argon for 30 min to remove the oxygen. The polymerization reaction was carried out at 60 °C for 20 h. After the reaction, a part of the micelle solution was used for several measurements.

Micelle Characterization. The size and shape of the polymeric micelle were characterized by the DLS measurements. The polydispersity factor (PDF: μ/Γ^2) was used for estimation of distribution of the obtained micelles, where μ is the second moment of the distribution of the relaxation rates and Γ is the intensity-weighted mean relaxation rate. The ratio μ/Γ^2 is a measure of the width the intensity distribution of relaxation rates, which can be used to give an indication of the polydispersity of the sample under investigation. The confirmation of the aldehyde group on the micelle surface was carried out using HPLC measurements with an aldehyde selective probe (Cascade Blue hydrazide, tripotassium salt).

Results and Discussion

Synthesis of Acetal-PEG/PLA Block Copolymer with Polymerizable Methacryloyl Group at the PLA Terminus (Acetal-PEG/PLA-MA). For the synthesis of heterotelechelic block copolymers, both initiation and termination must be utilized for the functionalizations. For this objective, a living polymerization system must be employed. We utilized a living ring-opening polymerization of ethylene oxide (EO) followed by lactide (LA) to prepare the PEG/PLA living block. To introduce the functional group at the PEG chain end, an initiator-carrying functional group should be utilized. We selected an aldehyde group as a functional group because it is stable in aqueous media and easy to react with amine even in aqueous media. The initiator with an aldehyde group, however, cannot be used for the anionic polymerization of EO, because the growing species must be deactivated by the active hydrogen in the aldehyde group. Therefore, we employed potassium alcoholate possessing an acetal group as the initiator for EO polymerization because the acetal group can be easily converted to the aldehyde group by an acid treatment and remains stable during the anionic polymerization. For an ω-terminal functionalization, methacrylic anhydride was used as an electrophilic agent and added to the living block copolymer system (Scheme 1). From GPC analysis, after the EO polymerization initiated with PDP, the M_n and the molecular weight distribution (MWD) were 5 800 and 1.04, respectively. The MW of the obtained PEG was in good agreement

Scheme 1



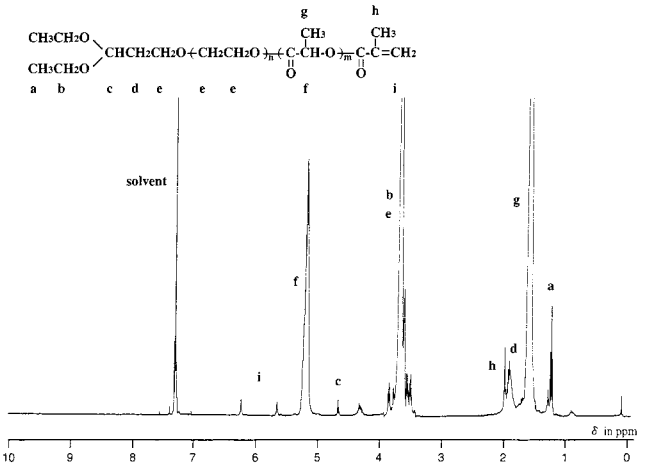


Figure 2. ^{1}H NMR spectrum of acetal-PEG/PLA-MA in $CDCl_3$.

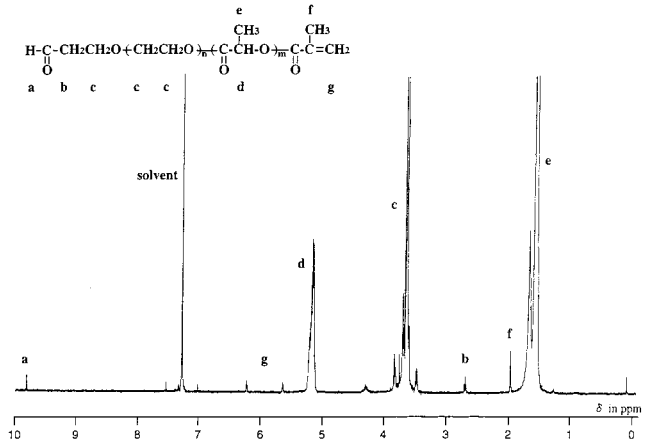


Figure 3. ^{1}H NMR spectrum of aldehyde-PEG/PLA-MA in $CDCl_3$.

with the initial monomer/initiator ratio. After the block copolymerization of LA, the M_n and MWD determined by the GPC data were 8 300 and 1.12, respectively. The MW of the PLA segment calculated by subtraction of the MW of the PEG segment from the total MW was only 2500, which was lower than the value expected by the initial monomer/prepolymer ratio. In the case of the GPC analysis of acetal-PEG/PLA block copolymers using DMF as the eluent, the block copolymer tends to appear on the rather lower MW side due to the adsorption of the block copolymer on the gel.

The segment length of the PLA in acetal-PEG/PLA block copolymers was estimated from the ^{1}H NMR spectrum. The assignments of the spectrum were carried out according to PEG/PLA block copolymers along with the initiator and the nucleophile and are shown in Figure 2. Signals based on both chain ends were clearly confirmed in the spectrum. Two signals at 5.7 and 6.2 ppm can be assigned to vinyl β-protons at the ω-chain end, while the acetal methine proton appears at 4.6 ppm. The M_n of the PEG segment determined from the ^{1}H NMR assuming one acetal group per each block copolymer agreed well with that from the GPC results. The integration ratio of the acetal methine proton versus the one of the vinyl β-proton was almost one. These two results indicate the quantitative preparation of heterotelechelic block polymers. From the methine protons of PLA and methylene protons of PEG segments, the MW of the PLA segment was determined to be 4000, which agreed with the initial molar ratio of the initiator versus LA.

Preparation of Aldehyde-PEG/PLA-MA Micelle. For the preparation of polymeric micelle, a dialysis method was employed.[10,11] Acetal-PEG/PLA-MA was dissolved in a good solvent for both segments such as DMF and then dialyzed against water. The conversion of the surface acetal groups into aldehyde groups was conducted directly after the micelle formation. The acetal-PEG/PLA-MA micelle solution (1.8 mg/mL) was adjusted to pH 2 with hydrochloric acid and stirred for 2 h at room temperature. After the pH of the mixture was neutralized with NaOH(aq), the solution was dialyzed against water to remove the salt. The conversion reaction of acetal into aldehyde was monitored by the ^{1}H NMR of the polymer after freeze-drying with water. The ^{1}H NMR spectrum of PEG/PLA after the hydrolysis reaction is shown in Figure 3. As can be seen in the figure, the end-aldehyde proton appears at 9.8 ppm, and the acetal methine proton around 4.6 ppm completely disappears, retaining two vinyl protons of the methacryloyl end appearing at 5.7 and 6.2 ppm. The extent of the conversion of the acetal group to the aldehyde group was determined by the ^{1}H NMR spectrum. More than 90% of the acetal was converted to aldehyde by the 2 h reaction. The size and the shape of the obtained polymeric micelle were estimated by the DLS measurement. Representative data for the gamma distribution of the obtained polymeric micelle are shown in Figure 4. The aldehyde-PEG/PLA-MA micelles thus obtained possess unimodal distribution in histogram analysis. The average diameter and polydispersity factor determined by a cumulant method were 34.8 nm and 0.09, respectively.

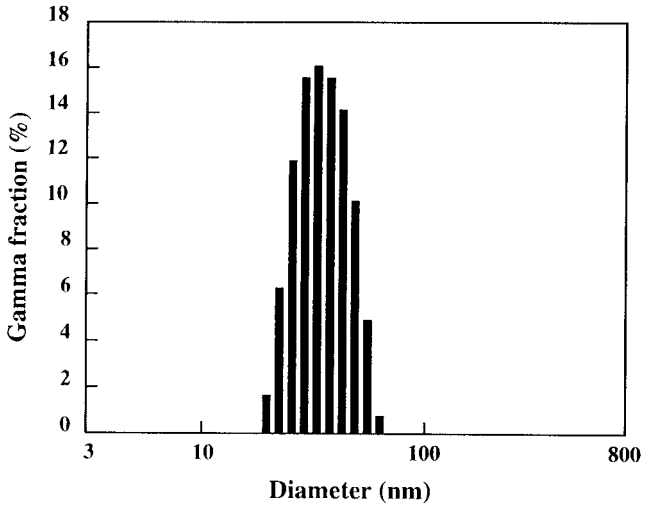


Figure 4. Gamma distribution of the aldehyde-PEG/PLA-MA micelle analyzed by DLS.

Polymerization of Methacryloyl Group in the Core of the Micelle. One of the main driving forces of polymeric micelle formation from the diblock copolymer is known to be the solvophobicity of one of the segments in the polymer in the selective solvent. The insolubility of one of the block segments in the media causes the segment to coagulate to form the core. The solvophilic segment in the block copolymer stabilizes the core as tethered chains from the core particle. A polymeric micelle thus formed has stable size and aggregation number. If the reactive groups on the polymeric micelle are utilized for conjugation with a certain molecule or substrate, however, its thermodynamic balance will be varied, because of the decreased entropy factor of the micelle shell chains due to the lowered mobility of the free end via the conjugation. To utilize such polymeric micelles, especially the reactive polymeric micelles, as a functional nanomaterials, the stabilization of the micelle must be one of the important objectives.

To stabilize the polymeric micelle, the dissociation of the block copolymer must be prevented. Polymerization (or cross-linking reaction) of each block copolymer in the micelle is one method for the stabilization. As previously stated, if the shell chains in the micelle are linked together (polymerized), the entropy factor becomes negative, which may work as the unstabilization of the micelle. Core polymerization is one of the most effective methods for the stabilization of the core−shell-type polymeric micelle.

To polymerize a micelle core, several methods can be envisaged, such as (1) entrapment of low molecular weight monomers in the core followed by polymerization, (2) introduction of the polymerizable group as the side chain of the core segment followed by the cross-linking reaction, and (3) introduction of the polymerizable group at the core segment end followed by the polymerization in the core.

The first method is based on the synthesis of the polymer or polymeric gel in the core to form a kind of semi-IPN core. Entanglement of the core segments to the formed gel stabilizes the particle. However, it still takes physical force to stabilize the micelle which may not show enough stability. There are several reports on the second method.[13−18] Liu et al. prepared poly(2-cinnamoylethyl methacrylate-*b*-acrylic acid) which forms polymeric micelles in water.[15,17] They cross-linked the

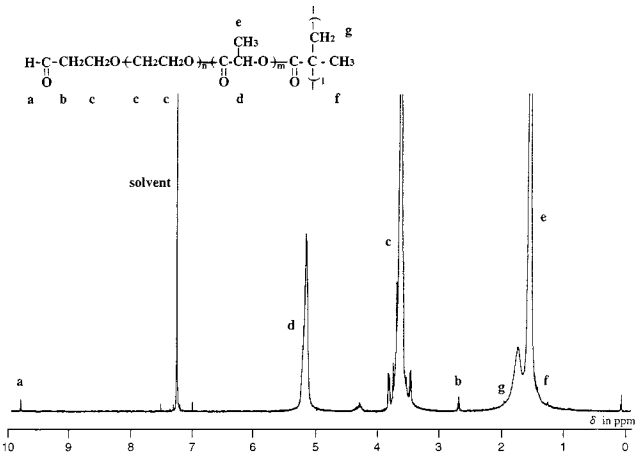


Figure 5. ^{1}H NMR spectrum of the aldehyde-PEG/PLA-MA micelle after core polymerization.

core segment by photochemically induced polymerization of the cinnamoyl side groups, which formed a very stable nanosphere. Though the cross-linking reaction using the side chains in the core segment improves the stability, at the same time it increases the core density, indicating a decrease in the free volume of the core. When the micelle core is envisaged as the container of a certain molecule such as a toxic drug, the decrease in the free volume should be avoided to improve the drug loading capacity.

To retain such free volume in the micelle core, the third method may be the best for the micelle stabilization. Ishizu et al. reported on the polymeric micelle formed by the microphase separation of AB-type block copolymer having polymerizable vinyl benzyl end group, followed by the polymerization of the end group.[19−28] The resulting particle was fairly stable. Instead of the microphase separation technique, we tried to polymerize the end group of the core segment in selective solvents.[12]

The core polymerization proceeded smoothly not only by conventional radical polymerization but also by the photopolymerization technique. The obtained micelle showed fairly high stability and maintained its small size and polydispersity. As anticipated, the core polymerized micelle showed excellent solubilization of rather larger molecules such as taxol.[12] In this experiment, we employed this method for the stabilized reactive micelle (nanoparticle).

To solubilize a radical initiator in the micelle core, a dispersion method was employed; viz. methylene chloride solution of AIBN was added to the prepared reactive micelle solution followed by the evaporation of methylene chloride at ambient temperature for 2 h. From our detailed investigation, this method is convenient and effective to introduce the initiator to the core. After oxygen was removed by the bubbling technique, the polymerization reaction was carried out at 60 °C for 20 h. The reaction mixture was transparent and homogeneous during the polymerization reaction, suggesting no aggregation or cross-linking reaction between the micelles. Actually, the resulting polymer was soluble in organic solvents after the freeze-drying of the reaction mixture. Figure 5 shows an ^{1}H NMR spectrum of the freeze-dried sample analyzed in $CDCl_3$. The original vinyl protons at 5.7 and 6.2 ppm completely disappeared, retaining the end-aldehyde proton intact at 9.8 ppm. This fact indicates the complete polymerization of the end methacrylate in the micelle core by the radical polymerization.

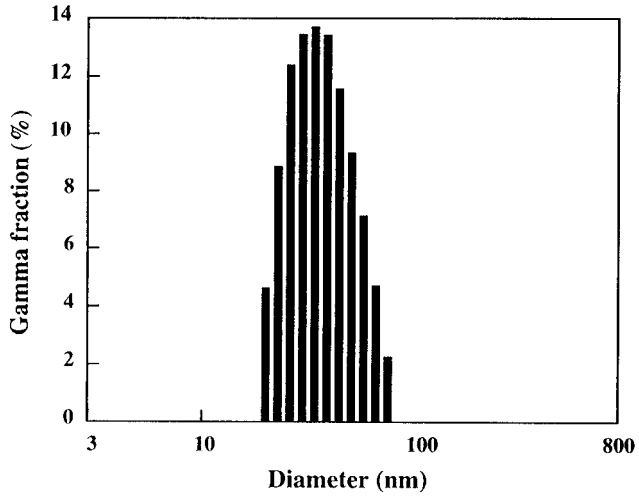


Figure 6. Gamma distribution of the aldehyde-PEG/PLA-MA micelle after core polymerization.

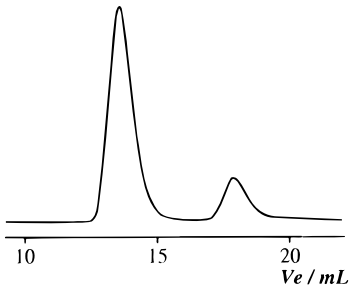


Figure 7. Gel permeation chromatogram in water of the aldehyde-PEG/PLA-MA micelle after core polymerization.

To obtain information on the size and shape of the polymerized micelle, DLS analysis was carried out. From the gamma distribution of the obtained micelle shown in Figure 6, it was confirmed that no remarkable change occurred in size and distribution after the core polymerization. The average diameter and PDF determined by a cumulant method were 34.8 nm and 0.08, respectively, which agreed well with those before polymerization. A GPC analysis also shows a polymeric micelle form as shown in Figure 7. The micelle peak appeared around V_e of 14 mL which was a symmetrical Gaussian shape without any shoulder, indicating no aggregation between micelles. The peak eluted in the low molecular weight region (V_e = 18 mL) was confirmed to be the PEG homopolymer without PLA segment which was contaminated in a very small amount during the block copolymerization process. This was confirmed by ^{1}H NMR analysis after a fractionation of the peak by GPC. After the fractionation of these peaks (14 and 18 mL in Figure 7), the sample weights were measured to be 3.0 and 0.1 mg, respectively. The extent of the contamination was thus less than 3% through the peak at 18 mL in Figure 7 was fairly larger due to the larger refractive index (RI) of PEG contamination than that of the polymeric micelle magnified the peak intensity. Actually, RI(PEG) was almost 20 times greater than RI(PEG/PLA micelle). On the basis of these results, the following is concluded: (1) the end vinyl groups were completely polymerized, (2) no aggregation took place between the micelles, and (3) most of the reactive aldehyde groups were retained during the stabilization procedure. The next task was to estimate the stability of the polymerized micelle.

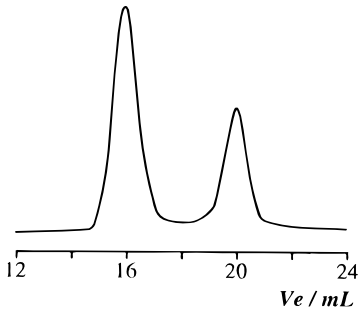


Figure 8. Gel permeation chromatogram in DMF of the aldehyde-PEG/PLA-MA micelle after core polymerization.

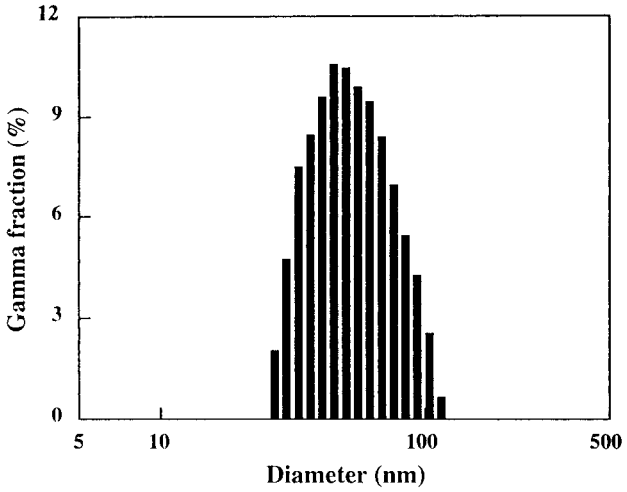


Figure 9. Gamma distribution in DMF of the aldehyde-PEG/PLA-MA micelle after core polymerization.

Stability Test of the Polymerized Micelle. Several experiments were carried out to estimate the stability of the polymerized micelle by means of GPC and DLS. As previously stated, the micelle after the core polymerization was soluble in several organic solvents including chloroform, THF, acetone, and DMF. Figure 8 shows a gel permeation chromatogram of the core-polymerized micelle in DMF as eluent. The pattern of the chromatogram was very similar to that by the aqueous GPC. The peak in the low-MW region should be small for the same reason as previously described. The peak in the high molecular weight region is somewhat peculiar. This polymerization system is not a core-cross-linking reaction but a vinyl polymerization of the block end group; therefore, a linear vinyl polymer (comb type) should be formed. Because DMF is a good solvent for both segments, the comb type poly(block polymer) should be observed, if dissociation of the micelle occurs in the organic solvent. In Figure 8, however, only the symmetrical Gaussian peak was eluted in the V_e of 16 mL, but no oligomeric peak could be seen in the lower MW region than the micelle. This fact indicates that no dissociation of the core-polymerized micelle took place even in the organic solvent probably due to the formation of interdigitated PLA loops in the micellar core. This was confirmed by the DLS analysis. The DLS analysis of the polymerized micelle in DMF showed unimodal distribution, and no oligomeric polymer was observed as shown in Figure 9. It is interesting to note that the average diameter of the micelle in DMF determined by a cumulant method was much larger than that in aqueous media, retaining a rather lower polydispersion factor. The average diameter and PDF of the micelle in DMF which were determined by a

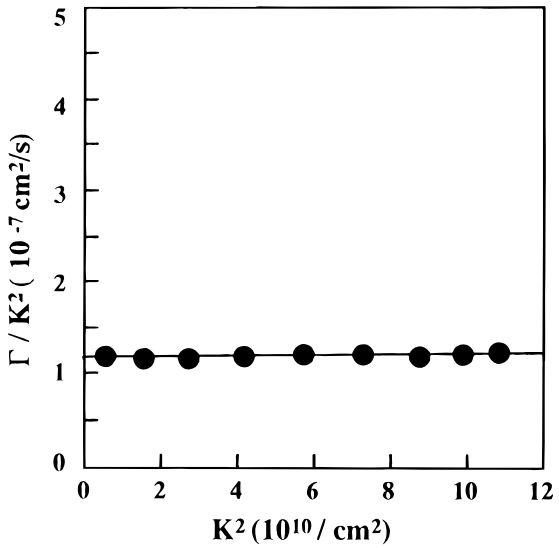


Figure 10. Plots of the K^2-scaled average characteristic line width Γ (Γ/K^2) versus K^2 for aldehyde-PEG/PLA-MA micelle after core polymerization at a 1.5 g L^{-1} and temperature of 25 °C.

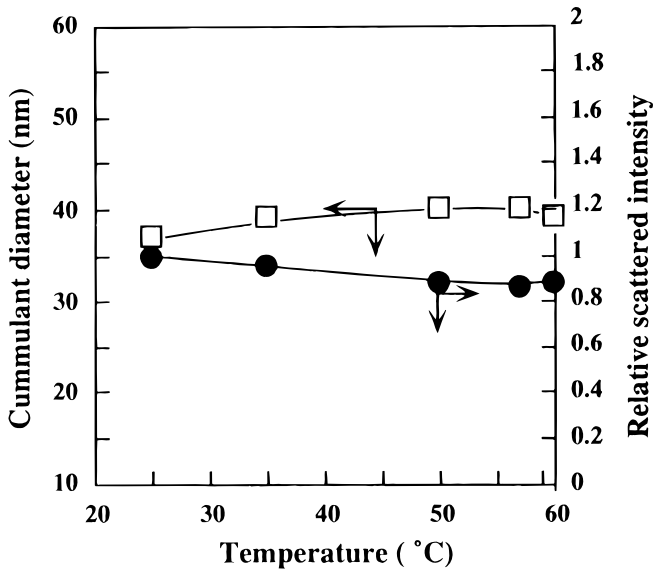


Figure 11. Temperature effects on the cumulant diameter of aldehyde−PEG/PLA−MA micelle after core polymerization (□) and the relative scattered intensity of aldehyde-PEG/PLA-MA micelle after core polymerization (●).

cumulant method were 55.1 nm and 0.114, respectively. The increased size (4 times by volume) in DMF can be attributed to the swelling of the PLA core of the micelle without any dissociation. The size of the micelle in methanol, which is a good solvent for the PEG segment and a poor solvent for PLA, was the same as that in water (35.6 nm). The formation of interdigitated PLA loops in the micellar core after the end group polymerization was tight enough and did not dissociate easily even in the core swelling. The formation of interdigitated loops in the micellar core may play an important role in core stabilization. The stabilization of the core-polymerized micelle was also confirmed by the interaction with the surfactant in aqueous media. When a low molecular weight surfactant such as sodium dodecyl sulfate (SDS) was added to a physically formed polymeric micelle (the same as that before core polymerization), the polymeric micelle collapsed almost completely.[12] On the contrary, the micelle solution after the core polymerization showed almost no change in its photon counting number under the same conditions though their size increased with increasing amount of added SDS. It is also suggested that the end-polymerized core can be a solubilized small molecular weight compound such as SDS. It is roughly estimated to be 3 times by volume. From this result, the possibility is suggested that this particle can be solubilized drug.

On the basis of these results, homopolymerization of the PLA end group in the core of the micelle proceeded completely, and the obtained nanoparticle was sufficiently stabilized.

Shape and Chemical Stability. To obtain detailed information on the micelle thus obtained, the angular dependency of the aldehyde-PEG/PLA-MA micelle after core polymerization was estimated from the DLS measurements. The dependence of the scaled characteristic line width on the scattering vector, which corresponds to the scattering angle, is shown in Figure 10. Even after the polymerization of the core of the micelle, no angle dependence of the scaled characteristic line width on the scattering vector was observed, suggesting the spherical structure of the micelle. To estimate the thermal stability of the micelle, the temperature effects on the cumulant diameter and the relative scattered intensity of aldehyde-PEG/PLA-MA micelle after core polymerization were carried out by DLS. In the temperature range between 25 and 60 °C, no change in any of the cumulant diameters and the relative scattered intensity of the micelle was observed. This indicated the high stability of the micelle at temperature changes in the range between 25 and 60 °C (Figure 11). The sphere was also stable in aqueous media at low temperature. After 3 months at 4 °C, no precipitate appeared in the micelle solution. In addition, the cumulant diameter and the relative scattered intensity of the micelle were almost the same value as those of the micelle just prepared. Therefore, the core-polymerized reactive micelle is fairly stable under these conditions.

Reactivity of Aldehyde Nanoparticle. The objective of this work was to create stable nanoparticles possessing reactive groups on the surface. As previously stated, the obtained particle was carrying an aldehyde group at the PEG chain end almost quantitatively. The next question was were the aldehyde groups located on the particle surface and could they be utilized for a conjugation reaction with a specific molecule. To confirm the reactivity of the aldehyde groups of the nanoparticle, a model reaction with a fluorescent (FL) probe was carried out. Cascade blue hydrazide tripotassium salt was chosen as the labeling reagent of the aldehyde groups because it rapidly reacts with aldehyde at ambient temperature in aqueous media. A micelle solution (c = 1.8 g/L, 1.0 mL) was stirred with about 10-fold molar excess of the fluorescence-active probe aqueous solution (c = 2.0 g/L, 0.5 mL) at room temperature for 20 min and then subjected directly to analysis by GPC equipped with a fluorescent detector. Polymerized micelle possessing a methoxy group at the PEG end was used as a control in the GPC analysis. Figure 12 shows the GPC diagrams of nanoparticles after FL probe treatment. The RI patterns in the aqueous GPC were the same as those previously mentioned as shown in Figure 7. A strong fluorescent signal was observed in the particle region in the case of the aldehyde micelle (Figure 12A), while no FL peak observed in the case of the methoxy micelle. This fact strongly indicated that

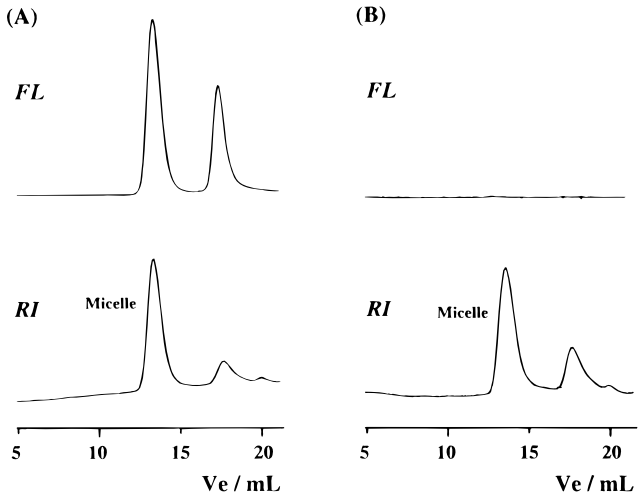


Figure 12. HPLC chromatograms of the aldehyde-PEG/PLA-MA micelle in the presence of Cascade blue hydradide tripotassium salt after core polymerization (A) and the methoxy-PEG/PLA-MA micelle in the presence of Cascade blue hydradide tripotassium salt after core polymerization (B).

the surface aldehyde groups reacted with FL probe efficiently. Further derivatization of the surface aldehyde group with specific molecules such as biotin, amino acid, and sugar molecules is now in progress and will be published elsewhere.

Conclusions

On the basis of all these results, it is concluded that aldehyde-PEG/PLA-MA micelle after core polymerization was quantitatively synthesized, and the micelle exhibits spherical core−shell-type structure in aqueous solution. The micelle also had the reactive aldehyde group on the surface and maintained high stability even in the presence of surfactants and in organic solvent. In addition, the micelle thus obtained was stable against temperature change and time passage. This functionalized micelle with high stability is expected not only to have wide utility in biomedical applications (including drug delivery, diagnosis, and surface modification through the coupling of bioactive substances) but also to be of great interest as a novel supramolecular architecture.

Acknowledgment. A part of this study was supported by a Grant-in-Aid for Scientific Research on Priority Area of "New Polymers and Their Nano-Organized Systems" (No. 08246249), from The Ministry of Education, Science, Sports and Culture, Japan. M.I. thanks the Japan Research Promotion Society for Cardiovascular Diseases for a scholarship to carry out this project.

References and Notes

(1) *Comprehensive Supramolecular Chemistry*; Lehn, J. M., Eds.; Pergamon: New York, 1996.
(2) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A., III *Angew. Chem., Int., Ed. Engl.* **1990**, *29*, 138.
(3) (a) Tuzar, Z.; Kratochvil, P. *Adv. Colloid Interface Sci.* **1976**, *6*, 201. (b) Riess, G.; Badahur, P.; Hurtrez, G. *Encyclopedia of Polymer Science and Technology*; Wiley: New York, 1985. (c) Gao, Z.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7353.
(4) Webber, S. E.; Munk, P.; Tuzar, Z. *Solvents and Self-Organization of Polymers;* NATO ASI Series E, Applied Science Vol. 327; Kluwer Academic: Dordrecht, 1996.
(5) Kim, Y. J.; Nagasaki, Y.; Kataoka, K.; Kato, M.; Yokoyama, M.; Okano, T.; Sakurai, Y. *Polym. Bull.* **1994**, *33*, 1.
(6) Cammas, S.; Nagasaki, Y.; Kataoka, K. *Bioconjugate Chem.* **1995**, *6*, 226.
(7) Nagasaki, Y.; Kutsuna, T.; Iijima, M.; Kato, M.; Kataoka, K. *Bioconjugate Chem.* **1995**, *6*, 231.
(8) Nagasaki, Y.; Iijima, M.; Kato, M.; Kataoka, K. *Bioconjugate Chem.* **1995**, *6*, 702.
(9) Nagasaki, Y.; Ogawa, R.; Yamamoto, S.; Kato, M.; Kataoka, K. *Macromolecules* **1997**, *30*, 6489.
(10) Scholz, C.; Iijima, M.; Nagasaki, Y.; Kataoka, K. *Macromolecules* **1995**, *28*, 7295.
(11) Nagasaki, Y.; Okada, T.; Scholz, C.; Iijima, M.; Kato, M.; Kataoka, K. *Macromolecules* **1998**, *31*, 1473.
(12) Kim, J. H.; Iijima, M.; Nagasaki, Y.; Kataoka, K., submitted for publication.
(13) Liu, G.; Qiao, L.; Guo, A. *Macromolecules* **1996**, *29*, 5508.
(14) Guo, A.; Liu, G.; Tao, J. *Macromolecules* **1996**, *29*, 2487.
(15) Henselwood, F.; Liu, G. *Macromolecules* **1997**, *30*, 488.
(16) Liu, G.; Smith, C. K.; Hu, N.; Tao, J. *Macromolecules* **1996**, *29*, 220.
(17) Liu, G. *Macromol. Symp.* **1997**, *113*, 233.
(18) Henselwood, F.; Liu, G. *Macromolecules*, in press.
(19) Ishizu, K.; Yukimasa, S.; Saito, R. *J. Polym. Sci. A, Polym. Chem.* **1993**, *31*, 3073.
(20) Ishizu, K.; Kuwahara, K. *J. Polym. Sci. A, Polym. Chem.* **1993**, *31*, 661.
(21) Ishizu, K.; Yukimasa, S.; Saito, R. *Polym. Commun.* **1991**, *32*, 386.
(22) Saito, R.; Ishizu, K.; Fukutomi, T. *Polymer* **1991**, *32*, 2258.
(23) Saito, R.; Ishizu, K.; Fukutomi, T. *Polymer* **1992**, *33*, 1712.
(24) Saito, R.; Ishizu, K.; Fukutomi, T. *Polymer* **1990**, *31*, 679.
(25) Ishizu, K.; Naruse, F.; Saito, R. *Polymer* **1993**, *34*, 3929.
(26) Saito, R.; Kotsubo, H.; Ishizu, K. *Polymer* **1994**, *35*, 1747.
(27) Ishizu, K. *Macromol. Rep.* **1995**, *A32*, 759.
(28) Ishizu, K.; Saito, R. *Polym. Plast. Technol. Eng.* **1992**, *31*, 607.

MA9815962