Synthesis of Comb-Type Biodegradable Polylactide through Depsipeptide−Lactide Copolymer Containing Serine Residues

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Introduction. The aliphatic polyester is one of the most widely utilized class of degradable polymers in the field of biomedical materials. Among them, poly-L-lactide, PLA, is the biodegradable and bioabsorbable polymer used clinically in wound closure,[1,2] tissue repair and regeneration,[3] and/or drug delivery.[4] PLA has a biodegradability, a good biocompatibility, a high mechanical strength, and excellent shaping and molding properties. However, PLA suffers from the difficulty of controlled degradation based on its high crystallinity and the induction of material defects based on lability of melt viscosity. Possible promising approaches to overcome these problems are the introduction of hydrophilic units to control the biodegradability and branched structure to stabilize the melt viscosity and/or to decrease the crystallinity in PLA. Many approaches, e.g., syntheses of block copolymer of PLA with poly(ethylene glycol) and terpolymer of PLA with polyhydroxyl compounds, were tried to control the degradation rate by varying the crystallinity.[5−13]

In general, it is well-known that a branched compound has different physicochemical properties compared with its linear counterpart owing to the molecular architecture. Long-chain branches predominantly affect the viscoelasticity of fluidity range, decreasing the viscosity and increasing the elasticity. On the other hand, short-chain branches predominantly affect the crystallinity. Since linear PLA, which has a higher glass transition point (T_g) (ca. 60 °C) and a higher melting point (T_m) (ca. 170 °C) than other aliphatic polyesters, is the thermoplastic and crystalline polymer, in order to take full advantage of these thermal characteristics, it is necessary to control the temperature in the melt-molding, stretching, and annealing processes. Therefore, the lower melt viscosity in a lower range of temperatures will be a major advantage for melt processing of PLA, such as for sutures[14] and bone fixation[15] in surgery.

In practice, as modifying PLAs, we reported previously the synthesis of random copolymers of L-lactide (LA) with depsipeptides consisting of glycolic acid (Glc) and α-amino acids [lysine (Lys) and aspartic acid (Asp)] having pendant amino or carboxyl groups,[16] poly[(Glc-Lys)-LA] and poly[(Glc-Asp)-LA], through ring-opening copolymerization of cyclo[Glc-Lys(Z)] and cyclo[Glc-Asp(OBzl)] with LA and subsequent deprotection of benzyloxycarbonyl (Z) and benzyl (Bzl) groups, respectively.[17] Furthermore, we reported the synthesis of graft copolymers composed of PLA and polysaccharides such as pullulan and amylose through the trimethylsilyl protection method.[18,19] The biodegradability of polymers obtained could be controlled by introduction of hydrophilic units and/or branched structure into PLA. Therefore, PLA having both hydrophilic units and branched structure can expect to be utilized as novel degradable materials.

In this paper, we report the synthesis of a novel comb-type biodegradable PLA having both hydrophilic units and branched structure. We could synthesize a novel comb-type PLA by graft polymerization of LA onto a depsipeptide−lactide copolymer containing serine residues. The obtained comb-type PLA is of interest as the biodegradable and bioabsorbable biomedical material.

Experimental Section. LA supplied by Wako Pure Chemical Co. (Tokyo, Japan) was recrystallized twice from ethyl acetate before using. DL-Polylactide (M_w = 10.6×10^4) was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Tin 2-ethylhexanoate and other chemicals were purchased from Wako Pure Chemical Co. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL GSX-400 using tetramethylsilane (TMS) or 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal reference. IR spectra were measured with a Perkin-Elmer 1600 series FTIR. The apparent molecular weight of polymer was estimated in tetrahydrofuran (THF) by gel-permeation chromatography (GPC) (column: TSK Gel ($G4000H_{XL}$ + $G2500H_{XL}$ + $G1000H_{XL}$); detector: UV_{254} or RI; standard: polystyrene). The molecular weight of comb-type PLA was measured by size-exclusion chromatography (SEC) using a dual-detector system, set in the direction of flow, consisting of a multiangle laser light scattering (MALLS) device and a differential refractometer in sequence. SEC−MALLS measurements were carried out at 40 °C in THF using Shodex GPC KF-806L × 5 columns, at the polymer concentration of 3 mg/mL and the flow rate of 1 mL/min. The MALLS device was a DAWN model F (Wyatt Technology Co.) where the laser beam of wavelength of 632.8 nm was focused on the 67 μL flow cell. T_g and T_m of comb-type PLA, poly[(Glc-Ser)-LA], and linear PLA were measured by a differential scanning calorimeter (DSC) (Rigaku TAS-200) using a heating rate of 10 °C/min.

1. Synthesis of Poly[(Glc-Ser)-LA]. The synthesis of poly[(Glc-Ser)-LA] was performed according to the method of Morita et al.[13,20] reported recently. Protected cyclodepsipeptide consisting of glycolic acid and *O*-benzyl-L-serine, cyclo[Glc-Ser(OBzl)], was prepared by the intramolecular reaction of *N*-bromoacetyl-Ser(OBzl) with $NaHCO_3$ in a large amount of *N*,*N*-dimethylformamide at 60 °C for 36 h. Poly{[Glc-Ser(OBzl)]-LA} was obtained by means of bulk polymerization of LA with cyclo[Glc-Ser(OBzl)] using tin 2-ethylhexanoate as an initiator.

Cyclo[Glc-Ser(OBzl)] (24 mg, 0.1 mmol), LA (130 mg, 0.9 mmol), and 90 μL of a freshly prepared 1.11×10^{-2} M solution of tin 2-ethylhexanoate in anhydrous THF (molar ratio of cyclo[Glc-Ser(OBzl)]/tin 2-ethylhexanoate = 1000) were added to a glass tube in a glovebox purged with dry argon. After removing the THF under vacuum and purging with dry argon, the tube was sealed in vacuo. The sealed tube was placed in an oil bath at 170 °C for 2 min and subsequently at 135 °C for 48 h. The

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Scheme 1. Synthetic Route of Comb-Type PLA

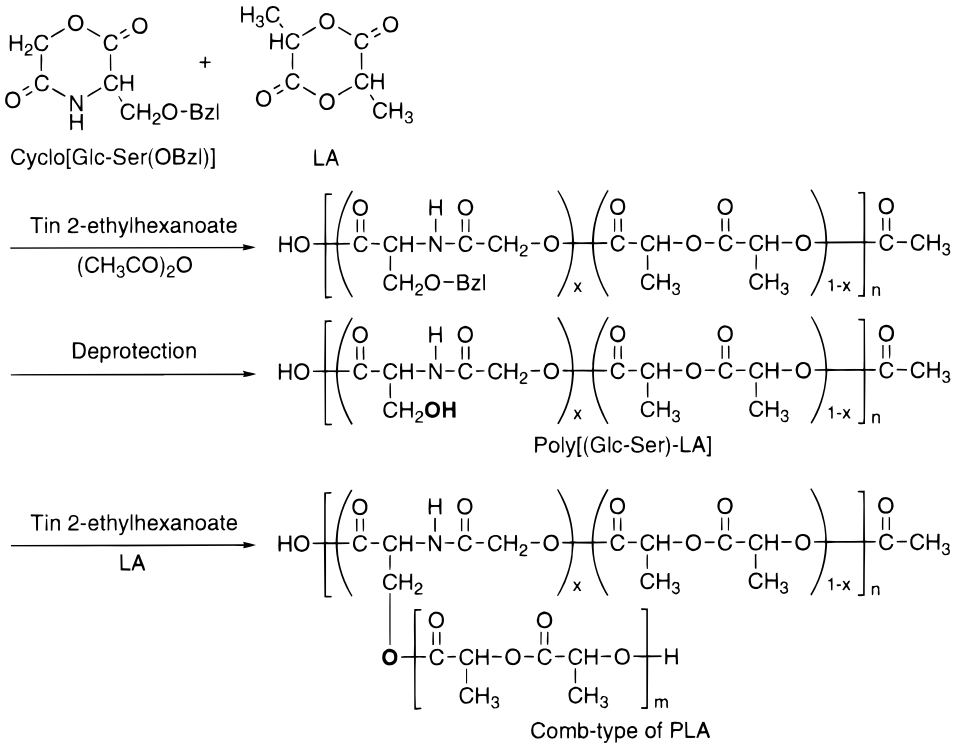

Table 1. Results of Graft Polymerization of LA using Poly[(Glc-Ser)-LA] as Macroinitiator[a]

LA		poly[(Glc-Ser)-LA][b]		comb-type PLA				
(mg)	(μmol)	(mg)	(μmol)	yield (wt %)	M_n^{GPC} ($\times 10^4$)	M_w/M_n^{GPC}	M_n^{MALLS} ($\times 10^4$)	M_w/M_n^{MALLS}
62.5	434	66.0	14.5	85.6	4.30	2.80	5.06	2.83

[a] Polymerization was carried out in bulk at 153 °C for 2 min and then at 130 °C for 4 h. Molar ratio of monomer to initiator (M/I) = 100. I: tin 2-ethylhexanoate. [b] Poly[(Glc-Ser)-LA] (mole fraction of (Glc-Ser) unit in copolymer = 4.2 mol %, $M_n = 1.11 \times 10^4$, $M_w/M_n = 2.66$) was used as a macroinitiator. GPC: estimated by GPC. MALLS: determined by SEC−MALLS.

reaction mixture was dissolved in a small amount of chloroform and poured into a large quantity of diethyl ether to precipitate poly{[Glc-Ser(OBzl)]-LA} as a light yellow solid. The content of the [Glc-Ser(OBzl)] unit in the copolymer was calculated to be 4.2 mol % from the ratio of the integration value of the 1.5 ppm ($-CH_3$) peak of LA to that of the 3.7−4.9 ppm (CH, CH_2) peak of Glc-Ser(OBzl) in ^{1}H NMR. The values of M_n and M_w/M_n were estimated to be 1.88×10^4 and 2.01 by GPC, respectively.

To achieve the deprotection, 130 mg of poly{[Glc-Ser(OBzl)]-LA} was treated with 10.0 mL of TFMSA−thioanisole/TFA at 0 °C for 30 min. The reaction mixture was poured into a large amount of diethyl ether to precipitate poly[(Glc-Ser)-LA] as a white solid. The results of IR and NMR spectra indicated the achievement of complete elimination of the benzyl group from the obtained poly{[Glc-Ser(OBzl)]-LA}. The GPC data of the obtained poly[(Glc-Ser)-LA] suggested that main-chain cleavage in these copolymers occurred slightly under our deprotection reaction condition using TFMSA−thioanisole/TFA.

2. Synthesis of Comb-Type PLA. Comb-type PLA was obtained by the following method. LA was graft-polymerized in bulk using tin 2-ethylhexanoate as a catalyst in the presence of poly[(Glc-Ser)-LA] with pendant hydroxy groups. The sealed tube prepared by the procedure described above was placed in an oil bath at 153 °C for 2 min, and then the reaction was allowed to proceed for 4 h at 130 °C. Purification was carried out simillar to that described above. IR (cm^{-1}, KBr disk): 3504 (OH), 3445 (CONH), 1758 (CO). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.59 (t, 3H, CH_3), 5.16 (q, 1H, C*H*CH_3), 4.30−4.50 (m, 2H, CHCH_2O), 4.60−4.70 (m, 1H, CH), 4.95−5.00 (m, 2H, OCH_2CO), 6.93 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ (ppm) = 16.7 (CH_3), 69.0 (*C*HCH_3), 169.6 (*C*OCHCH$_3$O). Other small peaks assigned to depsipeptide unit could not be detected.

Results and Discussion. The bulk graft polymerization was carried out in the presence of poly[(Glc-Ser)-LA] using tin 2-ethylhexanoate at 130 °C to give comb-type PLA as shown in Scheme 1. The results of graft polymerization are shown in Table 1. The graft polymerization was performed under a rigorously anhydrous condition. Poly[(Glc-Ser)-LA] used as a macroinitiator was carefully dried to avoid an initiation by water. The GPC trace of graft polymer, comb-type PLA, was symmetrical and unimodal, suggesting that no mixture of graft and linear polymer was produced. In practice, it has recently been reported that the energy of activation of an initiation involving alcohols such as polyols is obviously lower than that of neat tin 2-ethylhexanoate.[12,21] However, GPC analysis is not the method of choice to determine the molecular weight, since it always underestimates the molecular weight of the grafted polymer due to its smaller hydrodynamic volume in solution, compared with the case of linear polystyrene as a reference material. Therefore, comb-type PLA was analyzed by a combination of SEC and MALLS to characterize its effective molecular weight and hydro-

Table 2. Thermal Properties of Comb-Type PLA, Poly[(Glc-Ser)-LA], and Linear PLA

(co)polymer	M_n[a] ($\times 10^4$)	M_w/M_n[a]	Y[b] (mol %)	T_g[c] (°C)	T_m[c] (°C)	ΔH[c] (J/g)	X_c[d] (%)
comb-type PLA	4.30	2.80	1.6	40.4	135.1, 146.0	−20.5	21.9
poly[(Glc-Ser)-LA]	1.11	2.66	4.2	59.7	141.6, 151.6	−47.3	50.5
	1.04	2.25	9.5	55.3	133.3, 144.7	−21.4	22.8
linear PLA	1.80	1.81	0	62.1	166.6	−51.5	55.0
	6.30	2.37	0	63.8	173.5	−51.9	55.4

[a] Estimted by GPC. [b] Y: mole fraction of (Glc-Ser) unit in copolymer. [c] Determined by DSC. [d] X_c: crystallinity estimated from ΔH values.

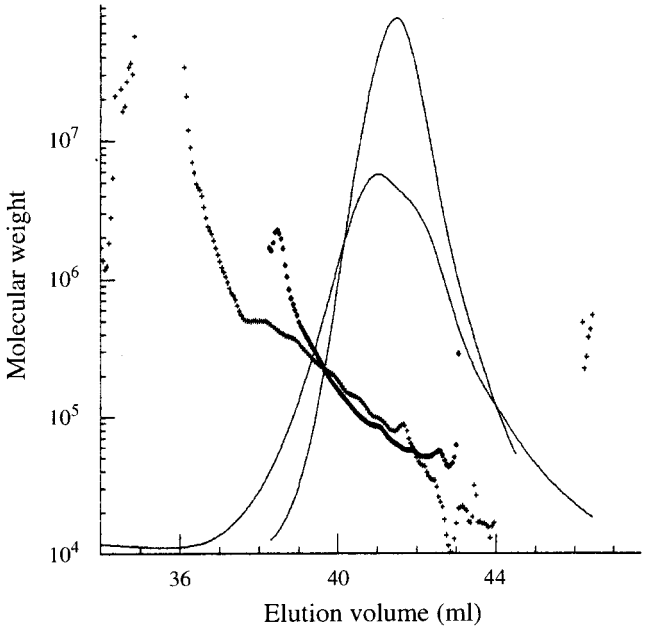

Figure 1. Comparison of comb-type PLA and linear DL-PLA by RI-monitored SEC curves and correlation of molecular weight vs elution volume: (+) comb-type PLA; (◆) DL-PLA.

dynamic volume in THF solution. Plots of molecular weight vs elution volume obtained from SEC−MALLS analysis suggested the hydrodynamic volume of comb-type PLA in THF solution was slightly small compared with that of linear DL-PLA having identical molecular weight (Figure 1). Since L-PLA was not dissolved in THF owing to its high crystallinity, that of comb-type PLA could not be directly compared with L-PLA having identical molecular weight. However, the result obtained above demonstrates clearly that the introduction of both hydrophilic units and branched structure highly contributes to the crystalline modification. Since the obtained comb-type PLA has ca. three branch points, plots of molecular weight vs hydrodynamic volume for comb-type PLA were observed to be slightly different compared with those for linear DL-PLA.

The results of thermal transition of comb-type PLA and poly[(Glc-Ser)-LA] of low content of (Glc-Ser) unit determined by DSC and their DSC curves are shown in Table 2 and Figure 2, respectively. T_g and T_m values of poly[(Glc-Ser)-LA], which is a random copolymer, decreased with increasing the content of the hydrophilic (Glc-Ser) unit. When the content of the (Glc-Ser) unit in random copolymer exceeded 15 mol %, the random copolymer became amorphous. The presence of the serine residues at relatively low percentage disrupted the crystalline region slightly, causing the decrease of T_m and the appearance of two melting endotherms as shown in Table 2. This quite small additional peak appeared at ca. 10 °C lower temperature than the dominant peak; as the serine content in the copolymer increased, the crystalline region was eventually disrupted entirely, producing a complete amorphous polymer. On the other hand, that of comb-type PLA decreased with introducing branched structure despite increasing the average molecular weight. The apparent crystallinities (X_c) of comb-type PLA and poly[(Glc-Ser)-LA] were calculated with the aid of the enthalpy of fusion of −93.7 J/g[22] for the perfectly crystalline PLA by the equation X_c (%) = $\Delta H/\Delta H_{theo} \times 100$. The results of X_c values shown in Table 2 suggested that the crystallinity of PLA could be easily controlled by the introduction of very low content of depsipeptide units with the OH group and/or branched structure. Therefore, it may be possible to change the degradation rate of the copolymer by controlling the crystallinity.

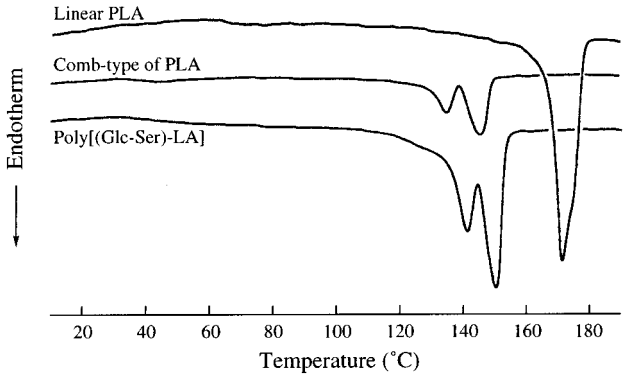

Figure 2. DSC curves of comb-type PLA, poly[(Glc-Ser)-LA], and linear PLA.

Biodegradability and/or molding properties can be expected to be easily controlled by varying the parameters such as distribution of hydroxy group, degree of branch, length of graft chain, and total molecular weight. We would like to report on the synthesis of such a variety of comb-type PLA and its properties (crystallinity, mechanical strength, biodegradability, biocompatibility, and so on) in a consequent paper.

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