Graft Polymerization of L-Lactide on Pullulan through the Trimethylsilyl Protection Method and Degradation of the Graft Copolymers

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Introduction. In the present communication we demonstrate the synthesis of a hybrid graft copolymer based on a combination of a hydrophobic poly(lactic acid) with hydrophilic polysaccharide as a new biodegradable material. Biodegradable polymers have become of interest not only from the standpoints of ecological and environmental situations but also for biomedical and pharmaceutical applications.[1,2] Based on their biodegradation properties, biocompatibility, high mechanical strength, and excellent shaping and molding properties, poly(lactic acid)s have been frequently applied as implantable carriers for drug delivery systems as well as surgical repair materials.[3−9] However, the high crystallinity of the polymers interferes with the controlled degradation, causes a decrease in compatibility with soft tissues as biomedical materials, and is an obstacle to applications as biodegradable soft plastics. Possible promising approaches to overcome these problems are introduction of hydrophilic segments and branched structure in poly(lactic acid)s. Many approaches, for example, synthesis of block copolymer with poly(ethylene glycol) and terpolymerization with polyhydroxy compounds, were carried out to control the degradation rate by varying the crystallinity.[10−17] Polysaccharides, such as amylose and pullulan, are typical examples of natural biodegradable hydrophilic polymers, which show enzymatic degradation behavior and relatively good biocompatibility. However, polysaccharides are insoluble in common organic solvents. Biodegradable polymers having both hydrophobic aliphatic groups and hydrophilic saccharide units in their main chains have been synthesized by polycondensation[18−21] or an enzymatic process[22,23] using mono- or oligosaccharides. Our group has tried to obtain graft copolymers of poly(lactic acid) and polysaccharides using the hydroxyl groups of the polysaccharides as initiating groups. However, these attempts were unsuccessful, because of the poor solubility of polysaccharides in organic solvents to give a heterogeneous reaction.

Poly(lactic acid)s are commonly synthesized by ring-opening polymerization of lactic acid dimer (lactide).[24,25] This ring-opening polymerization reaction can proceed in the presence of an alkali metal alkoxide to give poly(lactic acid) containing the alkoxy group as its terminal group.[13,14] In this paper, we report a new method to achieve graft polymerization of lactide on polysaccharide using mostly trimethylsilyl (TMS)-protected pullulan (TMSP). Using the protection technique via TMS groups, pullulan becomes soluble in organic solvents and the number of initiating groups, which means the number of grafted chains, can be controlled. As a result, the graft copolymer of pullulan having poly(lactic acid) side chains could be obtained by homogeneous graft polymerization of L-lactide on mostly TMS-protected pullulan in tetrahydrofuran (THF) by using potassium *tert*-butoxide (*t*-BuOK) as an initiator and consequent deprotection of TMS groups. The initial experiment for the evaluation of the graft copolymer as degradable materials and the hydrolytic degradability of films of the graft copolymer obtained were also investigated.

Experimental Section. Pullulan ($M_n = 1.5 \times 10^5$) was obtained from Hayashibara Co. (Okayama, Japan). Chlorotrimethylsilane (TMS-Cl) and *t*-BuOK were purchased from Kanto Chemical (Tokyo, Japan). L-Lactide, anhydrous THF, and other chemicals were purchased from Wako Pure Chemical (Tokyo, Japan). L-Lactide was recrystallized twice from ethyl acetate before using. The pullulan was dried at 60 °C under vacuum before using. Anhydrous THF was used without purification. Pyridine, *n*-hexane and other organic solvents were purified by the usual distillation method. Other regents were used without purification.

Trimethylsilylation of Pullulan. Pullulan was used after acidic degradation to enlarge the solubility and reactivity. The pullulan (4.86 g) was stirred in 40 mL of 1 M HCl aqueous solution at 95 °C for 5 min. After precipitation with MeOH, partially degraded pullulan (4.82 g) was obtained. The molecular weight of the partially degraded pullulan was estimated by GPC (column, Shodex KB-805; eluent, water; detector, refractive index (RI); standard, pullulan) to be $M_n = 4.4 \times 10^3$, $M_w/M_n = 1.5$. The partially degraded pullulan (1.00 g, 6.17 mmol of glucose unit) was suspended in pyridine (22.2 mL). TMS-Cl (7.03 mL, 56.4 mmol) was dissolved in *n*-hexane (16.6 mL), and the solution was added to the pullulan suspension. After 3 h of stirring, the reaction mixture was washed with saturated NaCl aqueous solution to remove pyridine hydrochloride. After reprecipitation with *n*-hexane and evaporation under reduced pressure, mostly trimethylsilylated pullulan (TMSP) was obtained. The introduction of TMS groups was confirmed by the methyl proton signal at $\delta = 0.10$ ppm besides the broad methyne and methylene proton signals of pullulan at $\delta = 3.5-5.5$ ppm on ^{1}H NMR spectra in $CDCl_3$. The molecular weight was estimated by GPC (column, Toso TSKgel G4000H_{XL} + G2500H_{XL}; eluent, THF; detector, RI; standard, polystyrene) with a data processing system (HITACHI D-2500) to be $M_n = 6.2 \times 10^3$, $M_w/M_n = 1.51$. The degree of trimethylsilylation (D_{TMS}) was estimated to be 90.4 mol %/hydroxyl group by ^{1}H NMR. Yield: 1.28 g (54.3%).

Graft Polymerization. The following procedures were basically carried out in a dry glovebox under an Ar atmosphere. TMSP (36.1 mg) was dissolved in dry THF (0.50 mL). *t*-BuOK (2.81 mg, 0.025 mmol) was dissolved in dry THF (0.50 mL), and the resulting solution was added to the TMSP solution. After stirring for 1 h, L-lactide (360.3 mg, 2.5 mmol) in THF (1.25 mL) was added. The polymerization was terminated when the reaction proceeded for 5, 15, 30, or 300 min by the addition of acetic acid (0.0014 mL) in THF (1.00 mL) to

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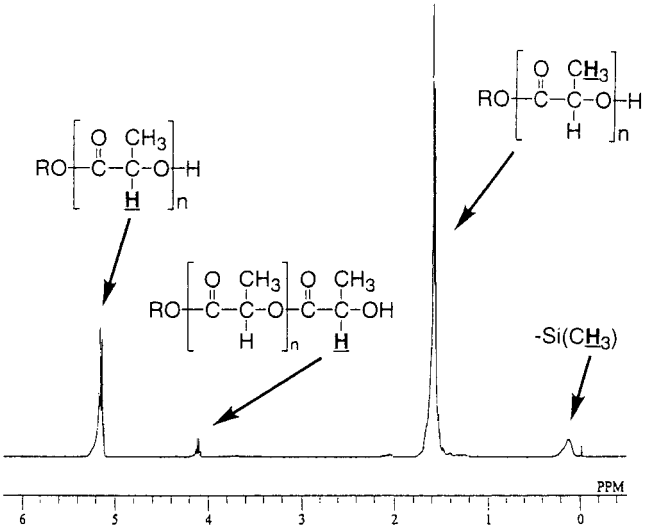


Figure 1. ^{1}H NMR spectra for TMS-protected graft copolymer (reaction time = 30 min).

the reaction mixture. The obtained products were precipitated twice with ethyl ether. Characterization of the TMS-protected graft copolymer was performed by ^{1}H NMR (Figure 1).

^{1}H NMR ($CDCl_3$): δ = 0.10 (s; Si(CH_3)$_3$), 1.55 (d; −CH(CH_3)-), 3.5−5.5 (broad, very weak; pullulan), 4.10 (q; −C*H*(CH_3)OH), 5.15 (q; −C*H*(CH_3)O−CO−).

The obtained TMS-protected graft copolymer was dissolved in 50 mL of $CHCl_3$, 250 mL of methanol was added, and the mixture was stirred for 2 days to deprotect the TMS groups. The deprotection of TMS groups was confirmed by the disappearance of the methyl proton signal at δ = 0.10 ppm by ^{1}H NMR. The broad and very weak methyne and methylene proton signals of pullulan existed at δ = 3.5−5.5 ppm. The molecular weights of the polymers were measured by GPC (column, Toso TSKgel G4000H_{XL} + G2500H_{XL}; eluent, THF; detector, RI; standard, polystyrene) with a data processing system (HITACHI D-2500).

^{1}H NMR ($CDCl_3$): δ = 1.55 (d; −CH(CH_3)−), 3.5−5.5 (broad, very weak; pullulan), 4.10 (q; −C*H*(CH_3)OH), 5.15 (q; −C*H*(CH_3)O−CO−). IR (KBr disk): 3000−3800 (−OH), 1730 cm^{-1} (C=O).

Hydrolytic Degradation. Poly(lactic acid) (M_n = 5.7 × 10^3) was obtained by a procedure similar to that described above without TMSP. The polymers (30 mg) were cast from chloroform solution (4 wt %) and dried overnight in vacuo to give colorless films having ca. 0.3 mm thickness. The films were incubated in 1/15 M-KH_2PO_4/$NaHPO_4$ buffer (pH = 7.0) at 37 °C for 16 days. At days 1, 2, 4, 8, and 16, the films were washed with distilled water and dried in vacuo. The molecular weights of the polymers were measured by GPC (column, Toso TSKgel G4000H_{XL} + G2500H_{XL}; eluent, THF; detector, RI; standard, polystyrene) with a data processing system (HITACHI D-2500). The hydrolysis rates were estimated by the molecular weight reduction (%) calculated with the equation shown in the caption of Figure 2.

Results and Discussion. Preparation of graft copolymer was carried out according to the procedure shown in Scheme 1. Most of hydroxyl groups of pullulan were protected by TMS groups to achieve solubility in organic solvents and to control the number of reaction sites with the alkali metal initiator. The degrees of

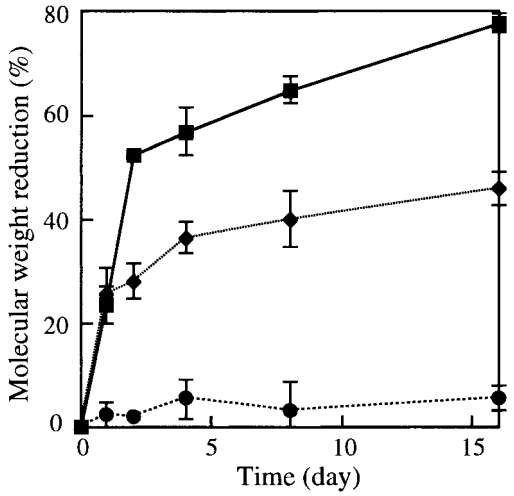
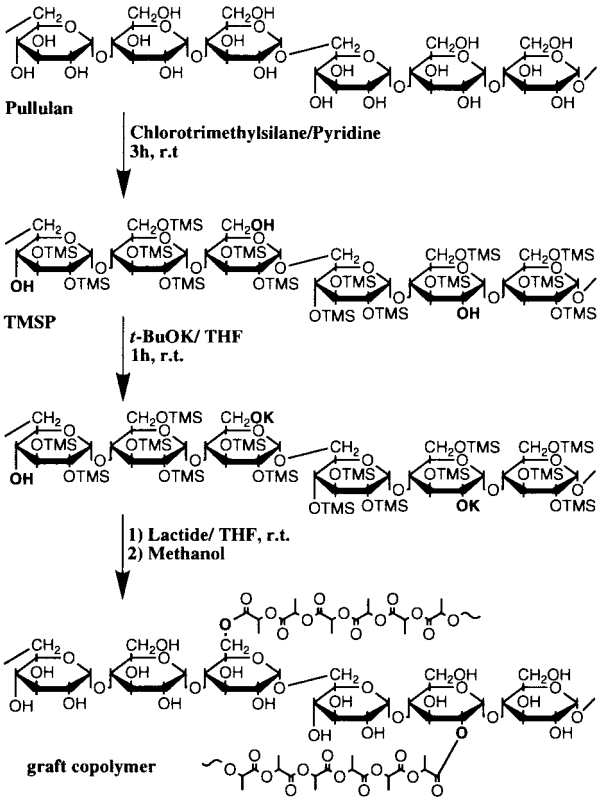


Figure 2. Degradation behavior of the graft copolymers and poly(lactic acid) in 1/15 M-KH_2PO_4/Na_2HPO_4 (pH = 7.0) at 37 °C in vitro: (◆) graft copolymer (content of sugar unit = 1.8 wt %); (■) graft copolymer (content of sugar unit = 3.4 wt %); (●) poly(lactic acid). Molecular weight reduction (%) = 100-($M_0 - M_t$)/M_0, where M_0 = initial molecular weight and M_t = molecular weight after degradation.

Scheme 1. Synthetic Route of Graft Copolymer



trimethylsilylation (D_{TMS}) of TMSP (M_n = 6.2 × 10^3, M_w/M_n = 1.51) were determined using ^{1}H NMR spectroscopy based on the area ratio of signals from methyl protons of TMS groups at δ = 0.10 ppm and protons of pullulan at 3.5−5.5 ppm in $CDCl_3$ to be 90.4 mol % per hydroxyl group. This value means that one free hydroxyl group exists per 3.5 glucose residues. On the basis of the molecular weight and D_{TMS} value, the net molecular weight of pullulan (unmodified pullulan) was caluculated to be 2.8 × 10^3. This value is smaller than that of partially degraded pullulan used for trimethylsilylation. Partial degradation of pullulan could

Table 1. Results of the Graft Polymerization of L-Lactide on TMSP[a]

run	reactn time (min)	yield (%)	10^{-4}Mn[b] (M_w/M_n)[b]	deg of polymerization of lactic acid[c]	content of sugar unit[d] (wt %)
1	5	43.9	8.3 (1.60)	74.0	3.4
2	15	44.0	10.5 (1.57)	96.4	2.7
3	30	46.0	15.1 (1.52)	140.9	1.8
4	300	43.6	8.3 (1.59)	70.4	3.4

[a] Polymerization was carried out with *t*-BuOK in THF at room temperture. Initial concentration of L-lactide = 1.0 mol/L; molar ratio of total OH group to *t*-BuOK = 12; molar ratio of L-lactide to *t*-BuOK = 100. [b] M_n: number-average molecular weight. M_w: weight-average molecular weight. Determined by GPC. [c] Number-average; calculated from ^{1}H NMR. [d] Content of sugar unit (wt %)

$$= \frac{\text{glucose unit (g)} \times 100}{\text{graft copolymer (g)}} = \frac{M_n \text{ of pullulan unit} \times 100}{M_n \text{ of graft copolymer}}$$

occur in the trimethylsilylation process, or high molecular weight products could be removed in the reprecipitation process. The obtained TMSP was soluble in THF, $CHCl_3$, and other common organic solvents. The polymerization reaction could be carried out homogeneously in THF with *t*-BuOK. Unprotected hydroxyl groups of TMSP were reacted with *t*-BuOK to be converted into the corresponding alkoxide in THF. The ring-opening polymerization of L-lactide was carried out in THF at room temperature using the polymer alkoxide as initiator. Figure 1 shows a typical example of ^{1}H NMR spectra of the TMS-protected graft copolymer obtained (content of sugar unit = 3.4 wt %, reaction time = 30 min). Signals of the methyl proton of the TMS group and poly(lactic acid) were observed at $\delta = 0.10$ and 1.55 ppm, respectively. The terminal and internal methyne proton signals of poly(lactic acid) were observed at $\delta = 4.10$ and 5.15 ppm, respectively. The methyne and methylene proton signals of pullulan were observed at $\delta = 3.5-5.5$ ppm but were broad and extremely weak, because the content of the sugar unit was very low.

The deprotection of TMS groups of the obtained graft copolymers was carried out by incubation in methanol and confirmed by the disappearance of ^{1}H NMR signals of methyl protons from TMS groups at $\delta = 0.10$ ppm. The sharp signals from poly(lactic acid) were still observed in ^{1}H NMR spectra. The methyne and methylene proton signals of pullulan were really observed at $\delta = 3.5-5.5$ ppm but were broad and extremely weak, because the content of the sugar unit was very low and the mobility of the sugar unit was very low in $CDCl_3$. All of the graft copolymers obtained were soluble in THF, chloroform, and dimethylformamide, but not soluble in water. The purity and molecular weight of the graft copolymers were investigated by GPC analysis (column, Toso TSKgel G4000H_{XL} + G2500H_{XL}; eluent, THF; detector, RI). A single and unimodal peak was observed around 12 min of retention time for the polymerization products, that is, TMS-protected graft copolymer. Although TMSP showed a single peak at 15 min of retention time, such a peak was not observed in the profile for the polymerization products. Contamination of poly(lactic acid) homopolymer was not observed in GPC analysis before and after deprotection of the TMS groups.

Table 1 summarizes the reaction conditions and the results of graft copolymerizations of L-lactide using TMSP. The molar ratio of total OH group (including TMS protected) to *t*-BuOK was 12, which means the molar ratio of free OH group to *t*-BuOK was 1.15. The molar ratios of L-lactide to *t*-BuOK in the feed were adjusted to 100. The initial concentration of L-lactide in the feed was adjusted to 1.0 mol/L. The degree of polymerization (P_n) of lactic acid was calculated using ^{1}H NMR spectroscopy on the basis of the area ratio of the terminal methyne proton signal at $\delta = 4.10$ ppm to the internal methyne proton signal at 5.15 ppm. There were no obvious differences in these values before and after deprotection. So, no degradation was observed in the TMS deprotection process. The molecular weight of the graft copolymer obtained, the yield, and the P_n of lactic acid tended to increase with increasing polymerization time, and the contents of the sugar unit (wt %) in the graft copolymer tended to decrease with increasing polymerization time, when the reaction time was no less than 30 min. However, when the reaction time was 300 min, the molecular weight of the graft copolymer, the yield, and the P_n of lactic acid were lower than the other results. This is probably because of the degradation of the poly(lactic acid) segment under a basic reaction condition. On the basis of the values in Table 1, the numbers of graft chains on each pullulan molecule were calculated to be 7.3−7.9 for the graft copolymers obtained by run 1 through run 4, which were almost constant. The contents of the sugar unit of the graft copolymer were low and the majority of the graft copolymers were poly(lactic acid). Therefore, these graft copolymers can be regarded as highly branched poly(lactic acid).

The hydrolysis behavior of the graft copolymers (content of sugar unit in graft copolymer = 1.8 and 3.4 wt %, reaction time = 30 and 5 min, respectively) was investigated in 1/15 M-KH_2PO_4/$NaHPO_4$ buffer (pH = 7.0) at 37 °C in vitro and compared with that of poly(lactic acid) (Figure 2). The degradation rates of the graft copolymers were significantly larger than that of poly(lactic acid). The graft copolymer having a higher sugar unit content (3.4%) showed a larger degradation rate than the graft copolymer having a lower sugar unit content (1.8%). The molecular weight reduction of the graft copolymer having a higher sugar unit content (3.4%) after day 20 could not be estimated because the hydrolyzed products became insoluble in both THF and water as the GPC eluent. This change can be attributed to the decrease of aliphatic poly(lactic acid) unit in the graft copolymer. Moreover, from the analysis of the hydrolyzed products by ^{1}H NMR, decrease of the P_n of poly(lactic acid) and the molecular weight reduction were observed, which can be attributed to the degradation of poly(lactic acid) side chain. These results all suggest that the introduction of a pullulan unit into poly(lactic acid)-based copolymer increases the degradation rate, and the hydrolytic degradation occurred at the poly(lactic acid) segment. The accelaration of degradation of the copolymer should be due to decreases in the crystallinity of the poly(lactic acid) segment by the introduction of a hydrophilic pullulan unit and its branched structure. The degradation rate of the graft copolymers became slow after day 2 or 4. These results suggest that the initial rapid degradation occurred in the low-crystallinity region of the graft copolymer; however, the degradation rate of the high crystallinity region of the graft copolymer was slow and should be similar to that of the poly(lactic acid) homopolymer.

In conclusion, we could synthesize new degradable graft copolymer composed of poly(lactic acid) and pullulan through a TMS protection technique. The obtained graft copolymers showed higher hydrolytic degradabilities compared with poly(lactic acid) by introduction of hydrophilic segments and branched structures. In these experiments, we obtained only a few graft copolymers having low sugar unit contents. However, the content of the sugar unit must be controlled by varying the amount of L-lactide in the feed and using TMSPs having various (higher) D_{TMS} values. We would like to report on the synthesis of such a variety of graft copolymers and their properties (crystallinity, mechanical strength, and so on) in consequent papers. This TMS protection method can be applied to various combinations of polysaccharides and aliphatic polyesters, poly(lactide, glycolide, or lactone). Such hybrid polymers are expected to be applied as novel biodegradable materials.

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