Branched Poly(lactide) Synthesized by Enzymatic Polymerization: Effects of Molecular Branches and Stereochemistry on Enzymatic Degradation and Alkaline Hydrolysis

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In this article the effects of the number of molecular branches (chain ends) and the stereochemistry of poly(lactide)s (PLAs) on the enzymatic degradation and alkaline hydrolysis are studied. Various linear and branched PLAs were synthesized using lipase PS (*Pseudomonas fluorescens*)-catalyzed ring-opening polymerization (ROP) of lactide monomers having different stereochemistries (L-lactide, D-lactide, and D,L-lactide). Five different alcohols were used as initiators for the ROP, and the monomer-to-initiator molar feed ratio was varied from 10 to 100 and 1000 for each branch in the polymer architecture. The properties of branched PLAs that would affect the enzymatic and alkaline degradations, i.e., the glass transition temperature, the melting temperature, the melting enthalpy, and the advancing contact angle, were determined. The PLA films were degraded using proteinase K or 1.0 M NaOH solution, and the weight loss and changes in the number average molecular weight (M_n) of the polymer were studied during 12 h of degradation. The results suggest that an increase in the number of molecular branches of branched PLAs enhances its enzymatic degradability and alkali hydrolyzability. Moreover, the change in M_n of the branched poly(L-lactide) (PLLA) by alkaline hydrolysis indicated that the decrease in M_n was in the first place dependent on the number of molecular branches and thereafter on the length of the molecular branch of branched PLA. The branched PLLA, poly(D-lactide) (PDLA), and poly(D,L-lactide) (PDLLA) differed in weight loss and change in M_n of the PLA segment during the enzymatic degradation. It is suggested that the branched PDLLA was degraded preferentially by proteinase K.

Introduction

Enzymatic polymerization has attracted considerable attention in both industrial and medical applications because of the nontoxic nature of the enzyme and the high selectivity and high activity of enzyme catalysts. For the last two decades, lipase-catalyzed polymerization has been investigated widely by several groups.[1−9] One of the notable advances in lipase catalysis was the discovery that some lipases reacted with alcohols not only in organic media but also at temperatures as high as 100 °C.[5,6] The polymerization of L-, D-, and D,L-lactides using lipase from *Pseudomonas fluorescens* (lipase PS) in the temperature range from 80 to 130 °C has been reported by Matsumura et al., and they obtained polylactide (PLA) with a molecular weight of 270 000 g/mol.[7,8] This was the first report of a synthesis of PLA using an enzyme as catalyst.

Branched PLA is different from linear PLA in its physical, thermal, and mechanical properties and is therefore interesting as a biodegradable and biocompatible polyester. Branched PLAs with different numbers of branches have been synthesized from L-, D-, and D,L-lactides using multifunctional alcohols, e.g., inositol, pentaerythritol, glycerol, and polyglycerine.[10−15] PLA with six molecular branches has been prepared using inositol as an initiator, and a number of catalysts were used for the ring-opening polymerization of the lactide.[10] The star-shaped poly(L-lactide) (PLLA) was synthesized using pentaerythritol or glycerol by Kim et al. and by Arvanitoyannis et al.[11,12] Kim et al. used stannous octoate or tetraphenyl-tin as a catalyst and suggested that pentaerythritol in conjugation with stannous octoate was an initiator for the living polymerization.[11] Korhonen et al. synthesized star-shaped PLLA using multifunctional alcohols with different numbers of hydroxyl groups as coinitiators.[13] Finne and Albertsson polymerized L-lactide using novel spirocyclic tin initiators and obtained linear and four-armed star-shaped PLLA.[14] Kricheldorf et al. polymerized L-lactide with $Bi(OAc)_3$ and pentaerythritol as the initiator and coinitiator, respectively.[15] Thus, various branched PLAs have been synthesized by organometallic catalysts, although an enzyme catalyst has never been used to prepare branched PLA.

The biodegradability of PLA has been studied by many groups.[16−23] PLLA and its copolymers have been reported to be degraded by different kinds of enzymes such as pronase, bromelain, *Rhizopus delemer* lipase, lipase from *Rhizopus arrhizus*, and proteinase K from *Tritirachium album*.[12,16−18] In particular, the enzymatic degradation of PLLA and its copolymers by proteinase K from *T. album* has been reported in several articles.[19−23] Reeve et al. carried out the degradation of a series

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of PLA stereo-copolymers by proteinase K and found that the enzyme preferentially degraded L-lactyl rather than D-lactyl units.[19] In addition, it was reported that the enzymatic degradation preferentially occurred in the amorphous region of PLA.[19] Cai et al. studied the effect of the crystallinity of PLA containing 96% L-lactyl and 4% D-lactyl units on the enzymatic degradation by proteinase K and concluded that the degradation rate increased with decreasing crystallinity.[20] Li and McCarthy studied the influence of crystallinity and stereochemistry on the enzymatic degradation of PLA and poly(D,L-lactide) (PDLLA) by proteinase K and showed that the degradation of PLA stereo-copolymers by proteinase K was accelerated if the crystallinity of the polymers was reduced and the hydrophilicity increased.[21] Vert et al. performed the enzymatic degradation of films of PLA stereo-copolymers by proteinase K and suggested that L−L, L−D, and D−L bonds were preferentially hydrolyzed over D−D bonds.[22,23] Hakkarainen et al. investigated two different biotic and abiotic degradations of PLA films and reported that the presence of low-molecular-weight lactic acid derivatives in films enhanced the degradability of PLA in the biotic medium.[24]

The alkaline and acidic hydrolyses of PLA have also been investigated. Makino et al. studied the effect of pH, temperature, and ionic strength on the hydrolysis of PLLA and PDLLA microcapsules[25] and found that the rate of decrease in molecular weight was lowest at around pH 5.0 and that it increased in both strongly acidic and strongly alkaline solutions. A higher ionic strength of the medium enhanced the degradation rate, and the rate of degradation was greater in an alkaline medium than in an acidic medium.[25] Hyon et al. examined the decrease in molecular weight and weight loss with respect to time of PDLLA films during hydrolysis in a phosphate buffer solution of pH 7.4 at 37 °C.[26] They reported that PDLLA films with higher molecular weights showed a longer retention of the initial properties such as molecular weight and tensile strength, and they confirmed that the hydrophilicity of the polymer influenced the hydrolysis.[26]

Enzymatic degradations and alkaline hydrolyses have been considered to occur at both the chain-end and the internal-chain bonds. Shih carried out the degradation of PDLLA by deuterochloric acid and suggested that the hydrolysis at the chain end was approximately 10 times faster than that of internal PDLLA bonds.[27] Hakkarainen et al. carried out the degradation of PLA films and found a more rapid molecular weight decrease and greater polydispersity in the biotic environment than in the abiotic environment. This indicated that degradation preferentially occurred near the chain ends in the biotic environment and that the ester bonds hydrolyzed randomly in the abiotic environment.[28] On the basis of these reports, it is suggested that branched PLA with a higher number of branches (chain ends) must show a higher degradability. The purpose of this work has therefore been to study the effect of the number of molecular branches of branched PLA on the enzymatic degradation and alkaline hydrolysis. We also aim to clarify the influence of PLA stereochemistry on the enzymatic and alkaline hydrolyses. Forty-five PLAs with different numbers of branches, different molecular weights, and different stereochemistries were synthesized by enzymatic polymerization using lipase PS. The properties of branched PLAs that are expected to have an effect on the enzymatic and alkaline degradation, i.e., the glass transition temperature (T_g), the melting temperature (T_m), the melting enthalpy (ΔH), and the hydrophobicity, were also determined. The branched PLA films were degraded using proteinase K and an alkaline solution. The effects of molecular branches and of the stereochemistry of the polymer on the enzymatic degradation and alkaline hydrolysis of branched PLAs are discussed.

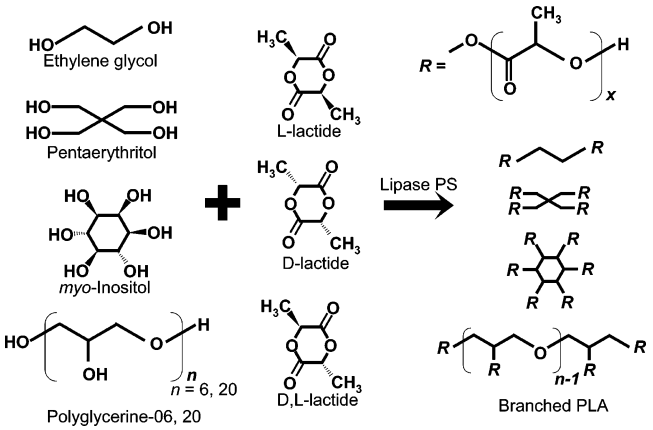

Figure 1. Reaction scheme of the enzymatic polymerizations in this study.

Experimental Section

Materials. L-, D-, and D,L-Lactides were purchased from Boehringer Ingelheim and kindly supplied by Purac and were recrystallized three times from toluene before use. Ethylene glycol (EG, Aldrich) was used after distillation. Pentaerythritol (Pen, Aldrich), myo-inositol (Ino, Sigma-Aldrich), polyglycerine-06 (PGL6, Daicel Chemical Industries), and polyglycerine-20 (PGL20, Daicel Chemical Industries) were used without further treatment. Amano lipase from *Pseudomonas fluorescens* (lipase PS) was purchased from Aldrich and dried for 2 days under vacuum at ~20 °C. Proteinase K from *T. album* (lyophilized powder, >30 units/mg protein) was purchased from Sigma-Aldrich and used without purification. The poly(L,L-lactide)s with number average molecular weight (polydispersity) of 1350 (1.10), 4580 (1.32), 7950 (1.27), 24 630 (1.33), and 40 400 (1.35) g/mol were purchased from Polymer Source, Inc., and used as references in some experiments.

Enzymatic Polymerization. Linear and branched PLA were prepared by ring-opening polymerization of either L-, D-, or D,L-lactides using lipase PS as a catalyst. Five multifunctional alcohols with different numbers of hydroxyl groups were used as initiators. A mixture of lactide (1.0 g), lipase PS (100 mg), and alcohol (appropriate amount) was reacted in an oil bath at 140 °C under a nitrogen atmosphere for 7 (inositol) or 5 days (the other alcohols). A silanized round-bottomed flask was used as the reaction vessel. Lactide, lipase, and alcohols were weighed and added to the vessel in a glovebox (Mbraun MB 150B-G-I) purged with nitrogen. The initiator/monomer molar ratio was set as 1:10, 1:100, or 1:1000 in each molecular branch. After the polymerization, the reaction mixture was dissolved in 10 mL of chloroform, and the insoluble enzyme was removed by filtration. Subsequently, the crude polymer was purified by precipitation (chloroform/methanol). In those cases where the polymer was soluble in methanol because of its low molecular weight, the polymer was obtained by evaporation of all solvent. The yields after purification were about 60%.

Enzymatic Degradation and Alkaline Hydrolysis. Films of the samples were prepared by casting of 0.5 mL of a solution of the polymer in chloroform (10 mg/mL) in a vial with an approximate diameter of 10 mm. The films were dried for 1 day under vacuum, and 0.1 M phosphate buffer (pH = 8.6) containing 0.2 mg/mL of proteinase K was then poured into the vial. The enzymatic treatment was carried out for 1, 3, 6, 9, and 12 h at 37 °C. The conditions of the enzymatic degradation were chosen as described in previous reports.[30,31] The alkaline hydrolysis of the films was performed in a vial filled with 1.0 mL of 1 M NaOH solution (pH = 14.0) at 37 °C. After the enzymatic degradation or the alkaline hydrolysis of the film, the film was washed with distilled water and dried for 1 day under vacuum, and the loss of

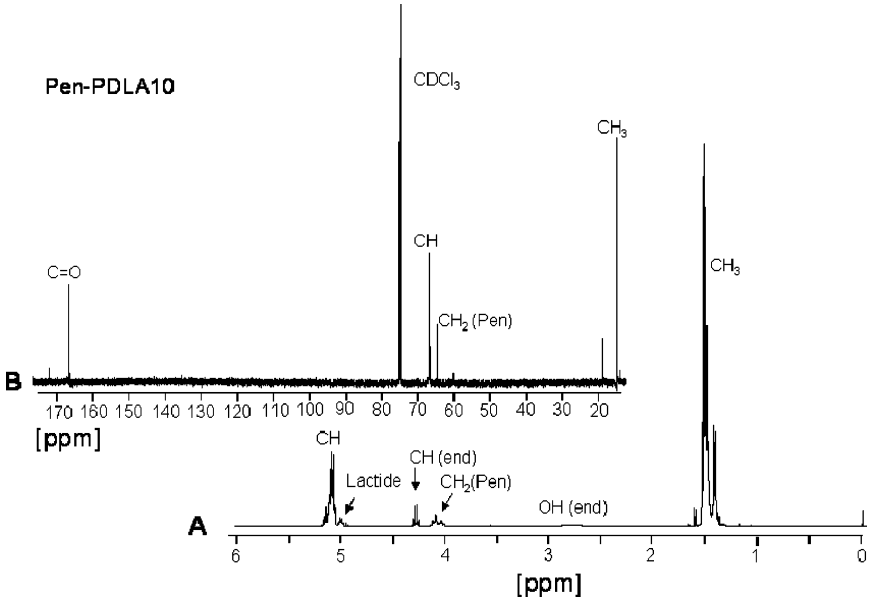

Figure 2. (A) ^{1}H and (B) ^{13}C NMR spectra of Pen-PDLA10 measured in $CDCl_3$.

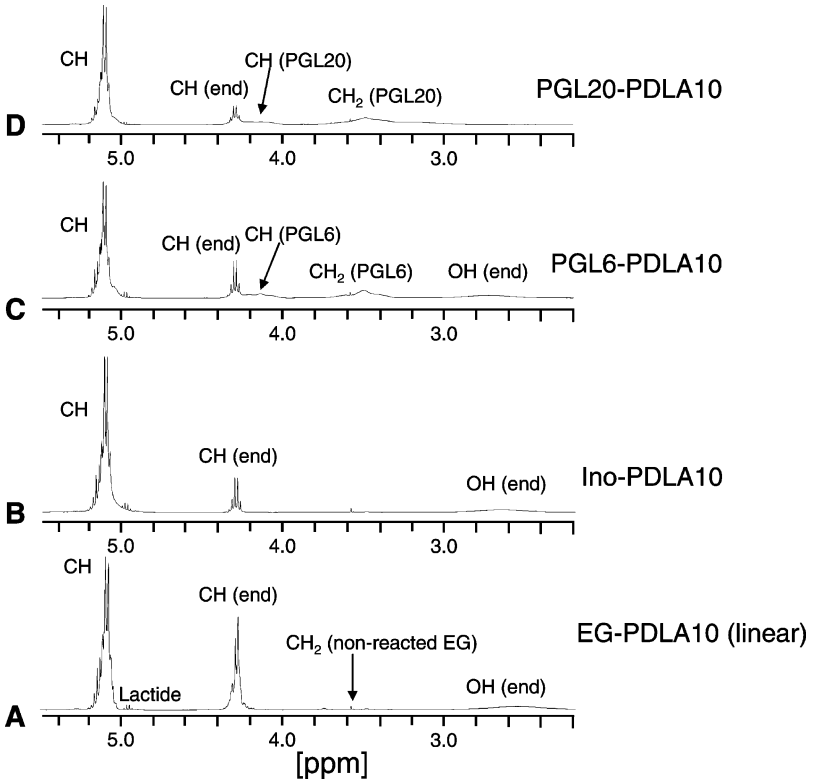

Figure 3. ^{1}H NMR spectra of (A) EG-PDLA10, (B) Ino-PDLA10, (C) PGL6-PDLA10, and (D) PGL20-PDLA10 measured in $CDCl_3$.

weight of the film was determined. After the enzymatic or alkaline treatment, the film was analyzed by ^{1}H NMR to determine the M_{n-NMR} of the PLA segment.

Characterization. The linear and branched PLAs synthesized by enzymatic polymerization were characterized by ^{1}H and ^{13}C NMR spectroscopies, size exclusion chromatography (SEC), and differential scanning calorimetry (DSC). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 and 100 MHz NMR instruments, $CDCl_3$ being used as the solvent and internal standard (δ = 7.26 and 77.0 ppm). The monomer conversion and the number average molecular weight (M_{n-NMR}) of the PLA segment (PLA molecular chain of each branch) were determined by ^{1}H NMR using δ = 4.4, 5.0, and 5.2 ppm peaks corresponding to the methine groups of the PLA chain end, lactide, and PLA, respectively. A Waters 717 plus autosampler and a Waters model 510 apparatus equipped with three PLgel 10 μm mixed B columns, 300 mm × 7.5 mm (Polymer Labs) were used to determine the molecular weight (M_{n-SEC}) and the polydispersity (M_w/M_n). M_{n-SEC} and M_w/M_n data were obtained by SEC calibrated with polystyrene standards in the 2000−90 000 g/mol range. Chloroform/methanol (95/5% v/v) was used as the eluent at a flow rate of 1.0 mL/min at 40 °C. Millenium software, version 3.20, was used to process the data. The glass transition temperature (T_g), the melting temperature (T_m), and the melting enthalpy (ΔH) were measured by DSC using a Mettler-Toledo DSC 820 module under a nitrogen atmosphere (nitrogen flow rate of 80 mL/min) with a sample mass of 5 ± 1 mg and a heating rate of 10 °C/min. The samples were subjected to a heating−cooling−heating cycle from −20 to 200 °C. ΔH was calculated as the difference between the melting and the crystallization enthalpies. The surface hydrophobicity of each thin film was estimated by the advancing contact angle (θ_{adv}) measurement with Milli-Q water using a contact angle and surface

tension meter (KSV Instruments Ltd.). Thin films of the samples were prepared on glass substrates by a solvent-casting technique from a solution of the sample in chloroform with a concentration of 1.0% (w/v), as previously reported.[29] The reported θ_{adv} value is an average of five measurements at different points.

Results and Discussion

Linear and branched PLAs were synthesized by enzymatic polymerization using five multifunctional alcohols, three lactide monomers of different stereochemistries, and three initiator/monomer ratios (1:10, 1:100, and 1:1000) in each molecular branch. Ethylene glycol (EG), pentaerythritol (Pen), inositol (Ino), polyglycerine (PGL6 and PGL20) (five alcohols), PLLA, PDLA, and PDLLA (three stereochemistries), and monomer-to-initiator ratios of 10, 100, and 1000 (three molecular weights) were used. For instance, EG-PLLA10 denotes the PLLA initiated by ethylene glycol with an initiator/monomer ratio of 1:10. In addition to the 45 synthesized PLAs, six purchased linear PLLAs with different molecular weights (M_n, 1350, 4580, 7950, 26 430, and 40 400 g/mol) were used as references in some experiments. The enzymatic polymerization by lipase PS is presented in Figure 1.

Enzymatic Polymerization. The molecular architectures of linear and branched PLA were investigated by ^{1}H and ^{13}C NMR spectra. Figure 2 shows the (A) ^{1}H NMR and (B) ^{13}C NMR spectra of Pen-PDLA10 measured in $CDCl_3$. No ^{1}H NMR peak arising from nonreacted hydroxyl groups of pentaerythritol was detected between 3.0 and 4.0 ppm, and this means that the number of molecular branches should be the same as the number of hydroxyl groups of the pentaerythritol. In addition, the ^{13}C NMR spectrum demonstrates the signal arising from methylene carbons of pentaerythritol. Thus, Pen-PDLA with a branched architecture was surely obtained. Similar results and assignments were achieved when the other multifunctional alcohols, such as ethylene glycol, inositol, polyglycerine-06, and polyglycerine-20, were adopted as initiators, as can be seen in Figure 3. In the case of Ino-PDLA10, no peak arising from the methine protons of inositol was observed in the ^{1}H NMR spectrum in Figure 3B, but the peak from methine carbons was recognized by ^{13}C NMR (Supporting Information). In the cases of branched PLLA and PDLLA, the same trends were observed; i.e., the NMR spectra showed that the enzymatic reactions between the multifunctional alcohols and the lactides were catalyzed by lipase PS.

The molecular weights of the samples were estimated by ^{1}H NMR and SEC, as shown in Table 1. ^{1}H NMR was used to calculate the M_{n-NMR} of the PLA segment by comparing the peak integrals of methine protons ($\delta = 5.2$ ppm, CH in Figure 2A) with those of methine protons next to the terminal hydroxyl groups ($\delta = 4.4$ ppm, CH(end) in Figure 2A). The PLA segment is equivalent to the PLA molecular chains of each branch; i.e., the M_{n-NMR} of the PLA segment denotes the number average molecular weight of each branch. For instance, the M_{n-NMR} of the PLA segment of Pen-PDLA10 was calculated as follows

$$[9.12\ (\text{the integral of CH}) + 1.00\ (\text{the integral of CH(end)})]/1.00\ (\text{the integral of CH(end)}) \times 72\ (\text{the molecular weight of a lactyl unit}) \approx 700$$

In each polymerization by lipase PS, the molecular weight of the samples increased with decreasing initiator concentration. These molecular weights did not however agree perfectly with the initiator/monomer ratio of the enzymatic polymerization, especially in the case of the 1:1000 of initiator/monomer ratio, because the conversion did not reach 100%, as shown in Figure 4.

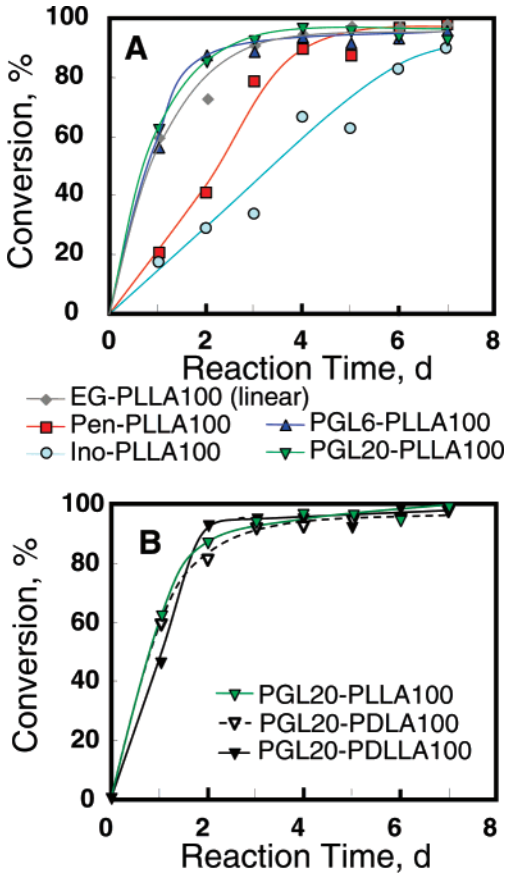

Figure 4. Dependence of conversions on reaction time at 140 °C under a nitrogen atmosphere for (A) five types of multifunctional alcohols and (B) three kinds of monomers.

The conversion of lactide was investigated by ^{1}H NMR by comparing the peak integrals of methine protons of PLA ($\delta = 5.2$ ppm) with those of lactide ($\delta = 5.0$ ppm). Figure 4 shows the conversion as a function of reaction time at 140 °C under a nitrogen atmosphere (A) for the different multifunctional alcohols and (B) for the three types of lactide. The conversion of Ino-PLLA100 (Figure 4A) reached over 90% after 7 days whereas the conversions of the other branched PLLA100s were much faster. Figure 4B shows that the three kinds of PGL20-PLA100 polymerized from L-, D-, and D,L-lactides hardly differed in the rate of conversion. On the basis of these results, the optimal polymerization times for the Ino-PLA and for the PLAs initiated with other alcohols were determined to be 5 and 7 days, respectively, independent of the stereochemistry of the lactide.

Thermal Properties. The T_g, T_m, and ΔH values of the polymers were determined by DSC, as shown in Table 1. Figure 5 shows the (A) T_g, (B) T_m, and (C) ΔH values of the linear and branched PLLAs as a function of the initial M_{n-NMR} of the PLLA segment. The T_g of linear and branched PLLAs with a molecular weight below 5000 g/mol was between 18 and 60 °C, and it increased with increasing M_{n-NMR} of the PLLA segment. The T_g of linear and branched PLLAs was found to be regulated by the M_{n-NMR} of the PLLA segment regardless of the M_{n-SEC} (whole molecular weight).

The T_m and ΔH of PLLA, Figures 5B and 5C, also increased with increasing M_{n-NMR} of the PLLA segment. The branched PLLAs with different numbers of branches showed almost the

Table 1. Properties of the Branched PLAs Used in This Study

monomer (polymer)	numbers of initiating OH groups	M/I[a]	M_{n-NMR} of PLA segment	M_{n-SEC} (M_w/M_n)	T_g (°C)	T_m (°C)	ΔH (J/g)	θ_{adv} (deg)
L-lactide (PLLA)	2 (EG)	10	300	1500 (1.1)				62 ± 8
		100	500	3600 (1.2)	22	89	10	66 ± 7
		1000	2300	7400 (1.3)	31	120	23	81 ± 1
	4 (Pen)	10	600	3500 (1.2)	18	61	3	55 ± 7
		100	2500	15 000 (1.1)	30	102	17	80 ± 3
		1000	3100	17 000 (1.1)	46	125	23	80 ± 4
	6 (Ino)	10	700	3300 (1.2)	36	52	5	74 ± 4
		100	2800	11 600 (1.2)	48	109	15	85 ± 4
		1000	4700	*b*	51	124	29	87 ± 4
	8 (PGL6)	10	400	4000 (1.2)	30			66 ± 8
		100	2300	15 400 (1.4)	47	103	20	78 ± 2
			3500	34 300 (1.1)	46	125	22	84 ± 4
	22 (PGL20)	10	700	10 800 (1.3)	26	94	3	65 ± 5
		100	2100	30 300 (1.2)	44	107	13	85 ± 4
		1000	4100	34 400 (1.5)	45	126	17	85 ± 5
D-lactide (PDLA)	2 (EG)	10	300	1700 (1.2)	12			67 ± 4
		100	1300	7900 (1.1)	51	122	29	86 ± 2
		1000	2200	8700 (1.3)	47	127	30	88 ± 1
	4 (Pen)	10	700	3300 (1.1)	28	118	3	79 ± 2
		100	2900	10 800 (1.3)	48	121	17	86 ± 2
		1000	4500	15 500 (1.1)	48	123	17	87 ± 1
	6 (Ino)	10	900	4600 (1.1)	34			82 ± 3
		100	4500	25 400 (1.1)	50	116	17	85 ± 1
		1000	8700	*b*	46	123	20	88 ± 1
	8 (PGL6)	10	600	8600 (1.2)	26	112	10	81 ± 4
		100	1800	23 100 (1.3)	47	123	17	85 ± 3
		1000	3600	26 300 (1.2)	48	131	26	88 ± 1
	22 (PGL20)	10	800	18 100 (1.2)	34			59 ± 1
		100	3000	34 000 (1.2)	47	124	15	88 ± 2
		1000	4600	36 700 (1.2)	43	130	24	88 ± 2
D,L-lactide (PDLLA)	2 (EG)	10	300	2200 (1.1)	18			56 ± 8
		100	900	2900 (1.4)	19			85 ± 6
		1000	3100	5300 (1.1)	19			85 ± 5
	4 (Pen)	10	900	2700 (1.1)	26			72 ± 4
		100	2100	6400 (1.8)	24			77 ± 1
		1000	3300	14 400 (1.2)	15			84 ± 4
	6 (Ino)	10	800	4100 (1.0)	14			76 ± 6
		100	2100	8200 (1.2)	24			79 ± 1
		1000	3300	*b*	28			80 ± 4
	8 (PGL6)	10	500	3200 (1.2)	16			67 ± 1
		100	1900	12 300 (1.2)	25			82 ± 3
		1000	2400	25 600 (1.1)	25			85 ± 3
	22 (PGL20)	10	600	9700 (1.3)	21			63 ± 4
		100	1800	25 700 (1.2)	17			83 ± 4
		1000	2900	36 300 (1.1)	13			84 ± 6

[a] Monomer-to-initiator ratio in each branch. [b] Ino-PLA with high molecular weight was not soluble in the mixed chloroform/methanol solvent.

same T_m and ΔH; these T_m and ΔH values were lower than those of the linear PLLA. The increase in the number of molecular branches reduced the T_m and ΔH values significantly, even when the polymer had the same M_{n-NMR} of the PLLA segment. For instance, the T_m and ΔH values of PGL20-PLLA were about 20 °C and 40 J/g, respectively, lower than those of the linear PLLA, as shown in Figures 5B and 5C. The M_{n-SEC} had less effect on the thermal properties of branched PLLA than the M_{n-NMR} of the PLLA segment.

It was also interesting to compare the thermal properties of the branched PLLA, branched PDLA, and branched PDLLA. The branched PDLA exhibited a trend similar to that of the branched PLLA in T_g, T_m, and ΔH, but the thermal properties of the branched PDLLA differed remarkably from those of the branched PLLA (Table 1). Branched PDLLA was amorphous and did not show any T_m. In addition, the T_g of the branched PDLLA was between 13 and 28 °C regardless of the number of molecular branches and the M_{n-NMR} of the PDLLA segment.

Surface Hydrophobicities of Thin Films. The surface hydrophobicities of the branched PLA thin films were assessed by determining the advancing contact angle (θ_{adv}) with Milli-Q water. Figure 6 shows θ_{adv} versus the M_{n-NMR} of the PLLA segment for thin films of PLAs with (A) five types of molecular branches and (B) three kinds of stereochemistries. The θ_{adv} values of linear and branched PLLA increased with increasing M_{n-NMR} of the PLLA segment, Figure 6A, and the θ_{adv} values of the linear and branched PLLAs with the same M_{n-NMR} of the PLLA segment were almost identical, indicating that the θ_{adv} values of the thin films were dependent on the M_{n-NMR} of the PLLA segment rather than on the M_{n-SEC}. Figure 6B

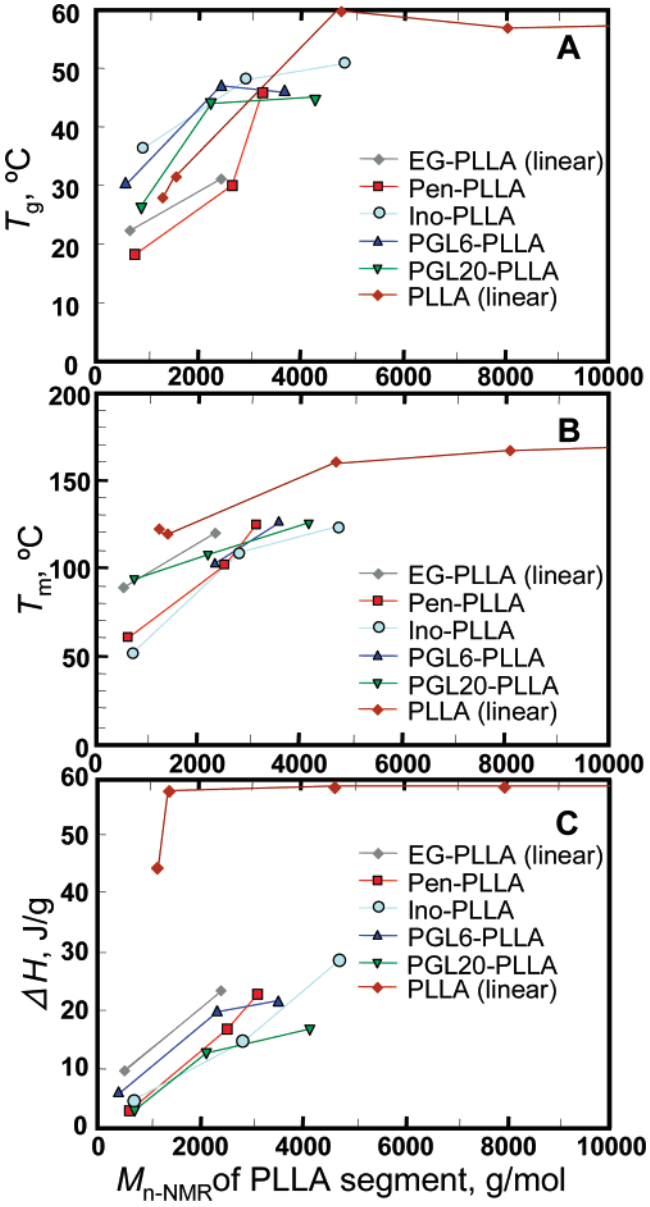

Figure 5. Effect of the M_{n-NMR} of the PLLA segment and molecular branches on (A) T_g, (B) T_m, and (C) ΔH of linear and branched PLLA as shown by DSC measurements.

indicates that PGL20-PLAs with different stereochemistries had almost the same θ_{adv} values. There was no difference in θ_{adv} value between the other branched PLLAs, i.e., Pen-PLLA, Ino-PLLA, PGL6-PLLA, and branched PDLA and PDLLA. In comparison to the linear PLAs, the branched thin PLA films were thus less hydrophobic at the surface, regardless of their stereochemistry.

Enzymatic Degradation. The linear and branched PLLA, PDLA, and PDLLA films were subjected to enzymatic degradation by proteinase K in a phosphate buffer solution (pH = 8.6) at 37 °C for 1, 3, 6, 9, and 12 h. The weight loss of the films was obtained according to eq 1

$$\text{weight loss } (\mu g/mm^2) = (W_{before} - W_{after})\ (\mu g)/(5^2 \times \pi)\ (mm^2) \quad (1)$$

where W_{before} is the initial weight before the degradation and W_{after} is the weight after the degradation and drying. The area at the bottom of the reaction vial is $5^2 \times \pi$ mm^2.

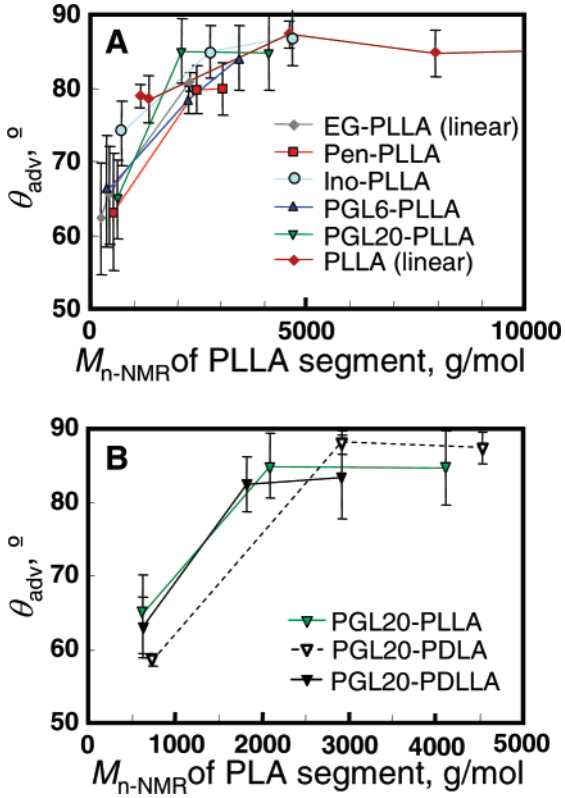

Figure 6. Effect of the M_{n-NMR} of the PLA segment and molecular branches on the advancing contact angles (θ_{adv}) of thin films on glass substrates for (A) linear and branched PLLA with different numbers of molecular branches and (B) three kinds of stereochemistries

Weight Loss and Molecular Weight Changes in PGL20-PLLA. The weight losses of PGL20-PLLA10 (M_{n-SEC}, 10 800), PGL20-PLLA100 (M_{n-SEC}, 30 300), and PGL20-PLLA1000 (M_{n-SEC}, 34 400) during 12 h are shown in Figure 7A, which shows that the weight loss from PGL20-PLLA during 12 h of enzymatic degradation increased with decreasing initial M_{n-SEC}.

It was also of interest to consider the changes in M_{n-NMR} of the PLLA segment during the 12 h enzymatic degradation period (Figure 7B). ^{1}H NMR was used to calculate the M_{n-NMR} value of the PLA segment during the degradation by comparing the peak integrals of methine protons (δ = 5.2 ppm, CH) with those of methine protons next to the terminal hydroxyl groups (δ = 4.4 ppm, CH(end)). This value, the M_{n-NMR} of the PLLA segment, is therefore the number average molecular weight of the PLLA segment of both branched PLLA and linear PLLA produced after the degradation. The M_{n-NMR} of the PLLA segment before the degradation shows the M_{n-NMR} of each branch. Figure 7B shows the time evolutions of the M_{n-NMR} of the PLLA segment for PGL20-PLLA10, PGL20-PLLA100, PGL20-PLLA1000, and EG-PLLA1000. In this figure, the M_{n-NMR} of the PLLA segment was constant during the enzymatic degradation. In addition, no peaks arising from the hydroxyl groups of polyglycerine-20 were detected by ^{1}H NMR after the enzymatic degradation (data not shown), implying that the PLLA segments were still attached to the hydroxyl groups. The results indicate that the enzymatic degradation for 12 h hardly reduced the M_{n-NMR} of the PLLA segment of the linear and branched PLLA, even though the films lost weight as shown in Figure 7A. In the cases of the other branched PLLAs, such as Pen-PLLA, Ino-PLLA, and PGL6-PLLA, the M_{n-NMR} of the PLLA segment also remained constant (data not shown). This means that the enzymatic degradation proceeds at the surface

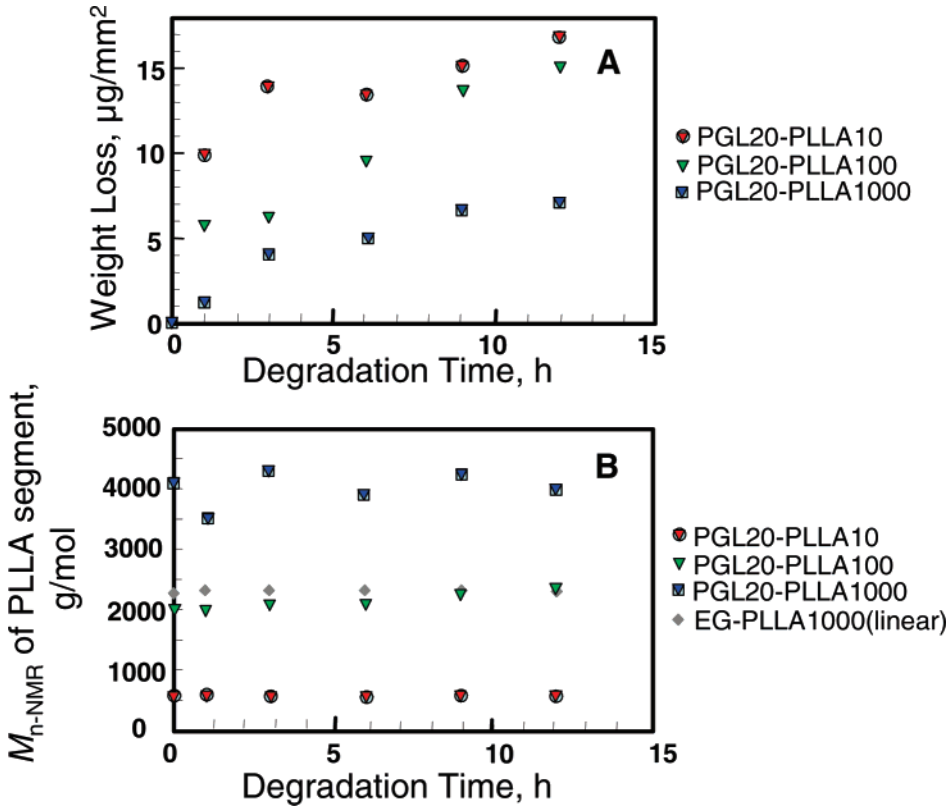

Figure 7. (A) Weight loss as a function of time for PGL20-PLLA10, PGL20-PLLA100, and PGL20-PLLA1000 and (B) M_{n-NMR} of the PLLA segment of PGL20-PLLA10, PGL20-PLLA100, PGL20-PLLA1000, and EG-PLLA1000 films.

of the PLLA films because the molecular weight of the bulk polymer, which is composed of almost the films, hardly changes.

Effects of Molecular Branches on Enzymatic Degradation. Figure 8 shows the weight loss in films of PLLAs with different numbers of molecular branches after enzymatic degradation for 12 h (A) as a function of the initial M_{n-NMR} of the PLLA segment and (B) as a function of initial M_{n-SEC}. The weight loss of the linear and branched PLLAs apparently decreased with increasing initial M_{n-NMR} of the PLLA segment (Figure 8A). In the relatively low-molecular-weight PLLA segment (below 2500 g/mol), the value of the weight loss may not be regulated by the initial M_{n-NMR} of the PLLA segment and by the number of molecular branches. When the molecular weight of the PLLA segment was greater than 2500 g/mol, the branched PLLA gradually approached the linear PLLA in weight loss with increasing M_{n-NMR} of the PLLA segment. In addition, the linear and branched PLLAs showed a similar trend in weight loss as a function of the M_{n-NMR} of the PLLA segment. The weight loss of the branched PLLA films was clearly regulated by the initial M_{n-NMR} of the PLLA segment rather than by the number of molecular branches.

Figure 8B also shows that the weight loss of linear and branched PLLAs apparently decreased with increasing initial M_{n-SEC}. Moreover, the branched PLLA, especially PGL20-PLLA, demonstrated a faster degradation than the linear PLLA. In the molecular weight range from 10 800 to 34 400 g/mol, PGL20-PLLA showed a higher weight loss than the linear PLLA by more than 15 μg/mm^2 over 12 h. This suggests that the less hydrophobic and less crystalline PLLA films were degraded faster. In contrast to the weight loss (16.9, 15.2, 7.1 μg/mm^2), the ΔH (3, 13, and 17 J/g), and θ_{adv} values (65°, 85°, and 85°) for PGL20-PLLA10, PGL20-PLLA100, and PGL20-PLLA1000 were hardly affected the degradation behavior. We suggest,

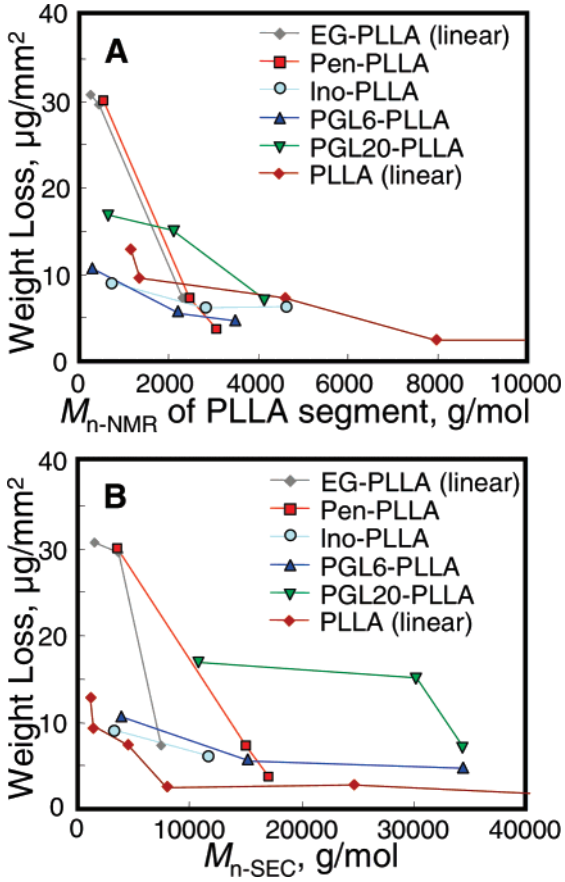

Figure 8. Weight losses of the linear and branched PLLA films after enzymatic degradation for 12 h as a function of (A) the initial M_{n-NMR} of the PLLA segment and (B) initial M_{n-SEC}.

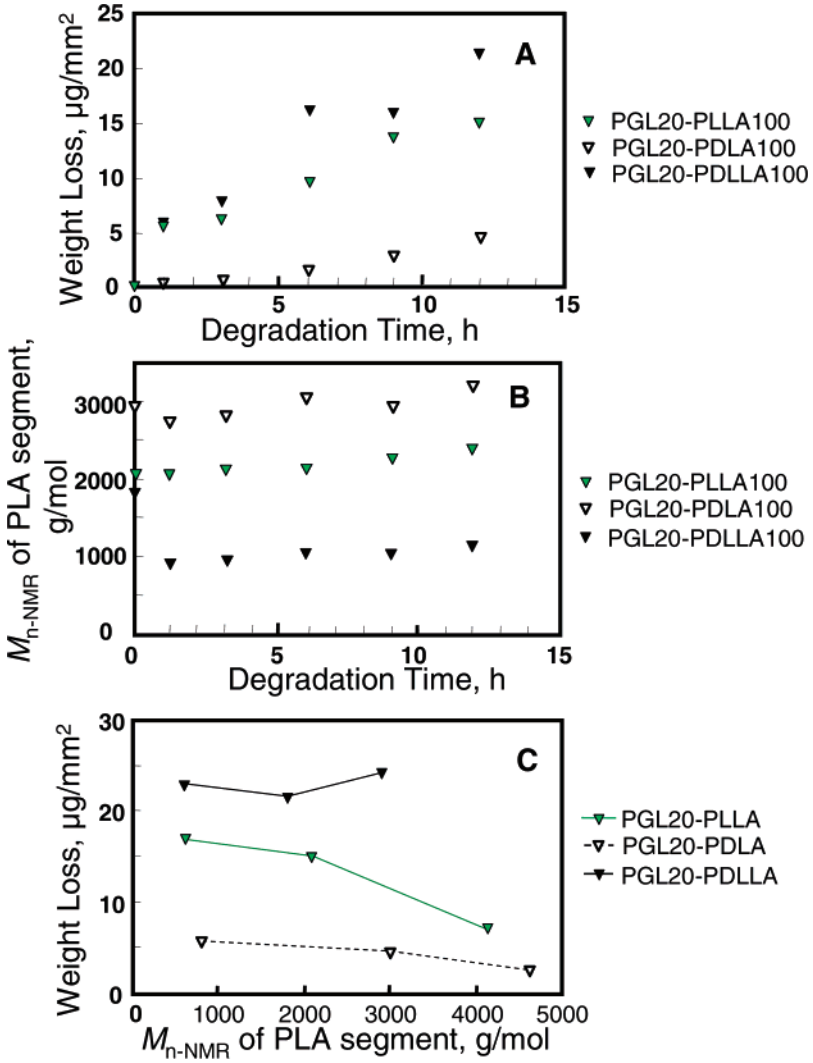

Figure 9. (A) Weight loss as a function of time for PGL20-PLA100 with different stereochemistries, (B) M_{n-NMR} of the PLA segment as a function of time for PGL20-PLA100 films with different stereochemistries during the enzymatic degradation, and (C) weight loss for PGL20-PLA films with different stereochemistries after the enzymatic degradation for 12 h as a function of the initial M_{n-NMR} of the PLA segment.

therefore, that the enzymatic degradation of branched PLLA film is affected by the number of chain ends rather than by the thermal properties and the surface hydrophobicity.

Effects of Stereochemistry on Enzymatic Degradation. The weight loss and the M_{n-NMR} of the PLA segment for PGL20-PLLA, PGL20-PDLA, and PGL20-PDLLA over 12 h are shown in Figure 9. Figure 9A shows that the weight loss during the enzymatic degradation for 12 h was in the following order: PGL20-PDLLA100 (M_{n-SEC}, 25 700) > PGL20-PLLA100 (M_{n-SEC}, 30 300) ≫ PGL20-PDLA100 (M_{n-SEC}, 34 000). This result implies that proteinase K preferentially degraded the PGL20-PDLLA, which contained D-lactyl units, indicating that the difference in degradation rate between PGL20-PDLLA and PGL20-PLLA depended on their ΔH or crystallinity. The result with regard to the degradation of PGL20-PDLA was expected, because the proteinase K has not been known to degrade the ester bond of D−D lactyl units.[22,23] The weight loss of the branched PDLA could be due only to the alkaline hydrolysis of PDLA chains by the buffer (pH = 8.6), since no peaks arising from the hydroxyl groups of polyglycerine-20 were observed by ^{1}H NMR after the enzymatic degradation (data not shown), implying that the PDLA molecular chains remained attached to the hydroxyl groups.

The change with time of the M_{n-NMR} of the PLA segment in PGL20-PLLA100, PGL20-PDLA100, and PGL20-PDLLA100 during the enzymatic degradation is shown in Figure 9B. The M_{n-NMR} of the PLA segment was almost constant except for the initial change in molecular weight of PGL20-PDLLA100. The PGL20-PDLLA showed the obvious degradation in the M_{n-NMR} of the PLA segment in addition to the weight loss because of their amorphous state. In the case of the PGL20-PDLA, the molecular branches should not be degraded because of the selectivity of proteinase K as indicated by the weight loss of PGL20-PDLA100 (Figure 9A).

Figure 9C shows the weight loss of films of PGL20-PLLA, PGL20-PDLA, and PGL20-PDLLA plotted against the initial M_{n-NMR} of the PLA segment. The weight loss of PGL20-PLLA and PGL20-PDLA decreased with increasing M_{n-NMR}, whereas that of PGL20-PDLLA remained constant. Generally speaking, amorphous polymers are hardly affected by molecular branches; therefore the M_{n-NMR} of the PDLLA segment in PGL20-PDLLA was not related to the thermal properties and hydrophobicity such as ΔH and θ_{adv} (Table 1). This was also the reason why the degradation behavior was not influenced by the molecular weight of the branched PDLLA.

Alkaline Hydrolysis. The linear and branched PLLA, PDLA, and PDLLA films were hydrolyzed in 1 M NaOH solution (pH = 14.0) at 37 °C for 1, 3, 6, 9, and 12 h, and the weight loss was calculated according to eq 1.

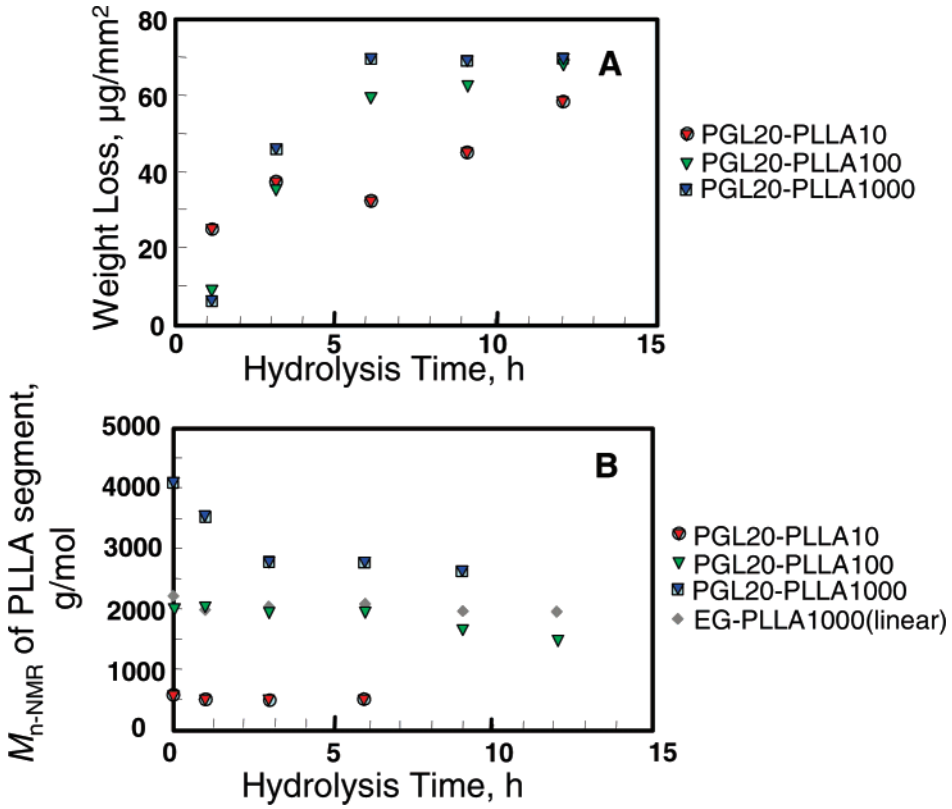

Figure 10. (A) Weight loss as a function of time for PGL20-PLLA10, PGL20-PLLA100, and PGL20-PLLA1000 and (B) M_{n-NMR} of the PLLA segment of PGL20-PLLA10, PGL20-PLLA100, PGL20-PLLA1000, and EG-PLLA1000 films.

Weight Loss and Molecular Weight Changes for PGL20-PLLA. Figure 10A shows the weight loss of the films of PGL20-PLLA10 (M_{n-SEC}, 10 800), PGL20-PLLA100 (M_{n-SEC}, 30 300), and PGL20-PLLA1000 (M_{n-SEC}, 34 400). The PGL20-PLLA1000 and PGL20-PLLA100 films suffered almost the same weight loss during 12 h, whereas the weight loss of PGL20-PLLA10 was less than that of the other two samples.

Figure 10B shows the change in M_{n-NMR} of the PLLA segment for PGL20-PLLA10, PGL20-PLLA100, PGL20-PLLA1000, and EG-PLLA1000 during the alkaline hydrolysis. The M_{n-NMR} of the PLLA segment of PGL20-PLLA, Figure 10B, apparently decreased. For instance, the M_{n-NMR} of the PLLA segment of PGL20-PLLA1000 decreased from 4100 to 2800 g/mol during 6 h of alkaline hydrolysis, while the M_{n-NMR} of the PLLA segment of PGL20-PLLA with a higher molecular weight decreased more rapidly during 12 h of alkaline hydrolysis, implying that the internal chain was hydrolyzed. However, the EG-PLLA1000 (linear) showed a small decrease in the M_{n-NMR} of the PLLA segment indicating that the chain end might be preferentially hydrolyzed. The molecular weight of the linear PLLA would show a decrease similar to that of the PGL20-PLLA if both the internal bonds and the chain ends of the PLLA were hydrolyzed. It is therefore suggested that the most important factor determining the rate of alkaline hydrolysis of branched PLLA is the number of molecular branches followed by the length of the molecular branches. Furthermore, the decreases of the molecular weights of PLA films during 12 h of alkaline hydrolysis mean that the internal and superficial hydrolyses proceed, as opposed to the enzymatic degradation of PLA films.

Effects of Molecular Branches and Stereochemistry on Alkaline Hydrolysis. Figure 11 shows the weight loss in of films of PLLAs having different numbers of molecular branches (A) as a function of the initial M_{n-NMR} of the PLLA segment and (B) as a function of M_{n-SEC}. Data for EG-PDLA, Ino-PDLA, PGL20-PDLA, EG-PDLLA, Ino-PDLLA, and PGL20-PDLLA are included to show the effect of the stereochemistry. Figures 11A and 11B show the same trend in weight loss for both linear and branched PLA; i.e., the weight loss for linear PLLA, Pen-PLLA, and Ino-PLLA decreased with increasing M_{n-NMR} of the PLLA segment and with increasing M_{n-SEC}, whereas the weight loss for PGL6-PLLA and PGL20-PLLA increased. In Figure 11A, the branched PLLAs with different numbers of branches hardly differed from the linear PLLAs with respect to weight loss, implying that the molecular branches did not affect the relationship between the weight loss by alkaline hydrolysis and the M_{n-NMR} of the PLLA segment. In Figure 11B, however, the branched PGL20-PLLA with high molecular weight had a significantly higher alkaline hydrolyzability than the linear PLLA.

The weight losses of PGL20-PDLA, PGL20-PDLLA, and PGL20-PLLA increased with increasing M_{n-NMR} of the PLA segment and with increasing M_{n-SEC}. Considering the data in Table 1, this result indicates that the weight losses of PGL20-PLLA1000, PGL20-PDLA1000, and PGL20-PDLLA1000 films during 12 h of alkaline hydrolysis (68.2, 65.5, and 47.8 μg/mm^2) were influenced by the number of molecular branches rather than by the T_g (45, 43, and 13 °C), ΔH (17, 24, and 0 J/g), θ_{adv} (85°, 88°, and 84°), or molecular weight. Furthermore, as can be seen in Figures 11A and 11B, Ino-PLLA, Ino-PDLA, and Ino-PDLLA showed a decrease in weight loss over 12 h with increasing M_{n-NMR} of the PLA segment and with increasing M_{n-SEC}, which is similar to the trends in weight loss for the linear PLLA, PDLA, and PDLLA. However, PGL20-PLLA, PGL20-PDLA, and PGL20-PDLLA demonstrated an increase in weight loss with increasing M_{n-NMR} of the PLA segment and with increasing M_{n-SEC}, indicating that the effect of molecular weight on the weight loss of PGL20-PLA was different from

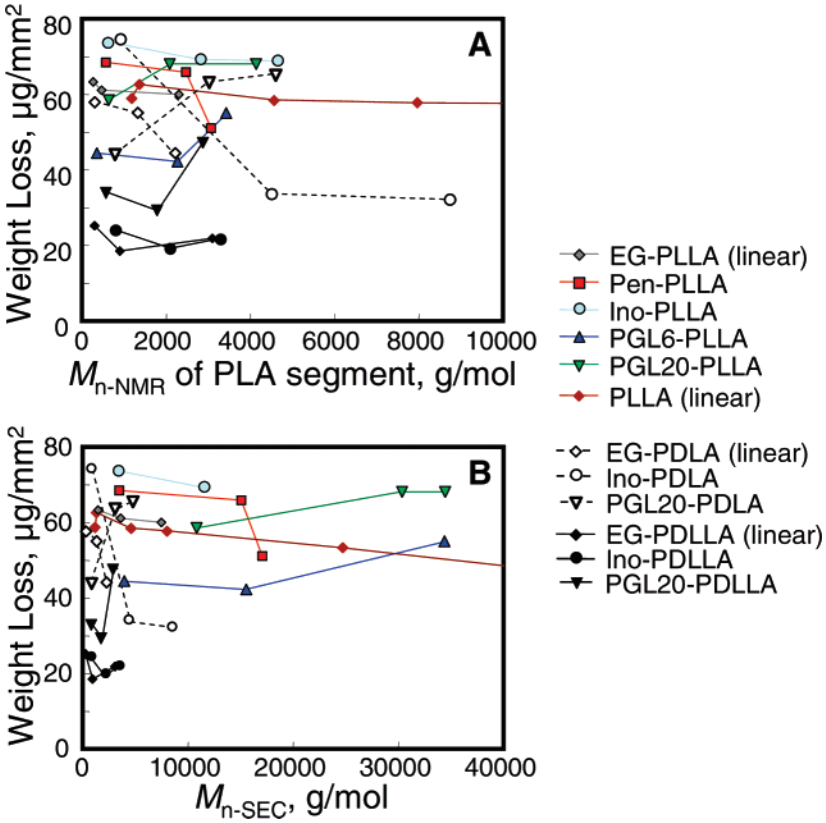

Figure 11. Weight losses of the linear and branched PLLA, PDLA, and PDLLA films after alkaline hydrolysis for 12 h as a function of (A) the initial M_{n-NMR} of the PLA segment and (B) the initial M_{n-SEC}.

that for linear PLA. In other words, no effect of the molecular branch on the alkaline hydrolysis was seen with six molecular branches, Ino-PLA, but an effect was seen in the polymer having 22 molecular branches, PGL20-PLA. Therefore, in contrast to linear PLA, PLA with many branches has the potential to show a high degradability as well as high mechanical properties, because a high molecular weight is necessary for high mechanical strength.

Conclusions

The number of molecular branches influences the enzymatic degradation and alkaline hydrolysis of PLAs. An increase in the number of molecular branches (chain ends) for branched PLAs enhanced the enzymatic degradability and the alkaline hydrolyzability when the M_{n-SEC} was similar. The initial M_{n-NMR} of the PLLA segment determined the weight loss of PLLA films during 12 h of enzymatic degradation by proteinase K, but the weight loss during alkaline hydrolysis was hardly affected by the initial M_{n-NMR} of the PLLA segment. PGL20-PLA with 22 molecular branches had a much higher alkaline hydrolysis rate and a different trend in the hydrolyzability compared with linear PLA, indicating that the molecular branching had no effect on the alkaline hydrolysis rate with the six molecular branches of Ino-PLA but that the 22 molecular branches of PGL20-PLA did have an effect. The data showing the M_{n-NMR} change of the PLA segment during alkaline hydrolysis indicate that the most important factor determining the rate of alkaline hydrolysis in the M_{n-NMR} of the PLA segment of branched PLAs was the number of molecular branches, followed by the length of the molecular branch. The influence of the stereochemistry of the branched PLA on both the enzymatic and alkaline hydrolysis was also studied. The branched PLLA, PDLA, and PDLLA differed in weight loss and in the M_{n-NMR} change of the PLA segment during the enzymatic degradation. It is suggested that the branched PDLLA was degraded preferentially by proteinase K. However, the alkaline hydrolysis of the branched PLAs was hardly affected by the stereochemistry.

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Supporting Information Available. Additional figures showing ^{13}C NMR spectra, as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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