Enzymatic Degradation of Poly(L-lactic acid) Fibers: Effects of Small Drawing

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ABSTRACT: The enzymatic degradation of poly(L-lactic acid) (PLLA) fibers with different low draw ratios (1.0, 1.2, and 1.4 times) was investigated in tris-HCl buffer solution (pH = 8.6) with proteinase K by the use of gravimetry, scanning electron microscopy (SEM), gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and tensile testing. Surprisingly, even the small drawings (1.2 and 1.4 times) disturbed the proteinase K catalyzed enzymatic degradation of the PLLA fibers. This should have been because the enzyme could not attach to the extended (strained) chains in the amorphous regions of the uniaxially oriented PLLA fibers or could not catalyze the cleavage of the strained chains. The accumulation of crystalline residues formed as a result of selective cleavage, and removal of the amorphous chains was not observed, even for as-spun PLLA fibers. This indicated the facile release of formed crystalline residues from the surface of the as-spun PLLA fibers during enzymatic degradation. Such release may have been because the crystalline regions of the as-spun PLLA fibers were oriented with their *c* axis parallel to the machine direction, as reported for biaxially oriented PLLA films. Gravimetry, SEM, and tensile testing could trace the enzymatic degradation of the PLLA fibers, although the enzymatic degradation of the PLLA fibers was untraceable by GPC and DSC. J Appl Polym Sci 103: 2064–2071, 2007

Key words: biodegradable; drawing; enzymes; orientation; polyesters

INTRODUCTION

Poly(L-lactic acid) (PLLA) is produced from renewable resources, mainly from the starch of various plants, and is widely used in biomedical, pharmaceutical, and commodity applications.[1–14] Recently, PLLA has been used in parts of automobiles and the housing of personal computers and mobile phones. The improvement of the mechanical properties of PLLA-based materials is a matter of concern, especially when they are used in commodity applications. One of the most effective methods for enhancing the mechanical properties of PLLA is orientation by drawing. A large number of articles have appeared on the fiber formation, orientation, and physical properties of PLLA fibers[15–52] and stereocomplex fibers from PLLA and its enantiomer poly(D-lactic acid).[53–55]

On the other hand, the uniaxial orientation of PLLA fibers has been reported to retard hydrolytic degradation in alkaline and neutral media without enzymes.[18,35] Also, the biaxial orientation[56] and uniaxial orientation[57] disturbs the enzymatic (proteinase K catalyzed) degradation of PLLA films. The crystalline residues formed as a result of biaxially oriented PLLA was found to be readily released from the surface of the materials.[56] However, as far as we are aware, there has been no study on the enzymatic degradation of PLLA in a fiber form where the pure effects of uniaxial orientation were investigated, although PLLA-fiber-based materials are expected to be used in numerous environmental applications.

The purposes of this study were to investigate the enzymatic (proteinase K catalyzed) degradation of PLLA fibers and to elucidate the pure effects of uniaxial orientation at low draw ratios on the enzymatic degradation of PLLA fibers. For these purposes, as-spun (i.e., draw ratio = 1 time) and uniaxially drawn PLLA fibers with different low draw ratios (1.2 and 1.4 times) were enzymatically degraded in the presence of proteinase K. The enzymatic degradation of the PLLA fibers was traced by gravimetry, scanning electron microscopy (SEM), gel permeation chroma-

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tography (GPC), differential scanning calorimetry (DSC), and tensile testing.

EXPERIMENTAL

Materials

As-spun and uniaxially drawn PLLA fibers with different draw ratios were kindly supplied by Unitika, Ltd. (Kyoto, Japan). The fibers were obtained by melt-spinning of PLLA (L-lactide/D-lactide = 98.8/1.2) at a spinneret temperature of 210°C and a rate of 3000 m/min and uniaxially drawn to low ratios of 1.2 and 1.4 times at 90°C. The as-spun fiber (i.e., draw ratio = 1 time) and the drawn fibers with draw ratios of 1.2 and 1.4 times were abbreviated as F1.0, F1.2, and F1.4, respectively. The diameters of the fibers were in the range 1.6–1.8 μm. The PLLA fibers were purified by extraction with *n*-hexane for 24 h at room temperature. *n*-Hexane was replaced once after about 10 min of immersion of the PLLA fibers. The fibers were dried under reduced pressure for 5 days.

Tris(hydroxymethyl)aminomethane (a specially prepared reagent, which was nuclease and proteinase tested), sodium azide (guaranteed grade), 0.1*M* HCl solution, and distilled water (high performance liquid chromatography grade) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and were used as received. Distilled water was used for the preparation of tris-HCl buffer solution and for the rinsing of degraded fibers. Proteinase K (lyophilized powder, 80% protein) was purchased from Sigma-Aldrich Co. (St. Louis, MO) and was used as received.

Enzymatic degradation

The enzymatic degradation of the fibers was performed according to the procedure reported by Cai et al.[57] and Reeve et al.[58] Namely, the fibers (ca. 50 mg) were placed in a vial filled with 5 mL of tris-HCl buffer solution (pH = 8.6) containing 1 mg of proteinase K (41 units/mg of protein) and 1 mg of sodium azide. The enzymatic degradation of the fibers was carried out at 37°C in a rotary shaker for periods up to 20 h. The degraded fibers were rinsed thoroughly with distilled water at 4°C to stop further degradation and were then dried under reduced pressure for at least 2 weeks for weight loss and physical measurements.

Measurements and observation

In the presence of proteinase K, it is known that PLLA specimens are degraded via a surface erosion mechanism.[59–62] Therefore, the weight loss per unit surface area of the degraded fibers [W_{Loss} (μg/mm^2)] and the percentage weight loss [W_{Loss} (%)] were calculated with the following equations with the weight of the fibers before degradation (W_{Before}), the weight of the fibers after degradation (W_{After}), and the fiber surface area before degradation (S_{Before}):

$$W_{Loss}(\mu g/mm^2) = (W_{Before} - W_{After})/S_{Before} \quad (1)$$

$$W_{Loss}(\%) = 100(W_{Before} - W_{After})/W_{Before} \quad (2)$$

The weight-average molecular weight (M_w), number-average molecular weight (M_n), and molecular weight distribution of the polymers were evaluated in chloroform at 40°C by a Tosoh (Tokyo, Japan) GPC system (RI-8020 refractive index monitor) with two TSK gel columns (GMH_{XL}) with polystyrene standards.

The glass-transition temperature (T_g), cold crystallization temperature (T_{cc}), melting temperature (T_m), enthalpy of cold crystallization (ΔH_{cc}), and enthalpy of melting (ΔH_m) of the fibers were determined with a Shimadzu (Kyoto, Japan) DSC-50 differential scanning calorimeter. The fibers (sample weight ≈ 3 mg) were heated at a rate of 10°C/min from room temperature to 200°C under a nitrogen gas flow at a rate of 50 mL/min. T_g, T_{cc}, T_m, ΔH_{cc}, and ΔH_m values were calibrated with benzophenone, indium, and tin as standards. The crystallinity (X_c) of the fibers was evaluated according to the following equation:[62,63]

$$X_c(\%) = 100(\Delta H_{cc} + \Delta H_m)/135 \quad (3)$$

where 135 J/g was ΔH_m of PLLA crystals having an infinite size, as reported by Miyata and Masuko.[64] By

TABLE I
Characteristics and Properties of PLLA Fibers Before and After Enzymatic Degradation

Code	Degradation time (h)	Draw ratio (times)	$M_n/10^5$ (g/mol)	M_w/M_n	X_c (°C)	T_g (°C)	T_{cc} (°C)	T_m (°C)	SA (%)[a]	YM (GPa)	TS (MPa)	EB (%)
F1.0	0	1.00	7.68	1.72	22.4	65.4	75.3	167.6	48	5.3	239	96.8
	20		7.13	1.88	24.6	62.7	74.1	165.3		0	0	0
F1.2	0	1.21	8.23	1.62	35.9	62.7		166.6	3	12.6	279	73.3
	20		8.51	1.44	36.5	60.6		166.4		7.3	256	32.8
F1.4	0	1.41	8.44	1.52	37.1	62.3		167.5	5	11.9	411	32.8
	20		8.76	1.43	37.5	60.0		167.0		13.7	421	27.0

YM = Young's modulus; TS = tensile strength; EB = elongation at break.
[a] Shrinkage after annealing at 70°C for 1 h.

definition, ΔH_{cc} and ΔH_m are negative and positive, respectively. The characteristics and properties of the PLLA fibers before and after the degradation are summarized in Table I. The morphology of the fibers was studied with a Hitachi (Tokyo) SEM (X-650). The fibers for SEM observation were coated with carbon to a thickness of about 20 nm.

The shrinkage or dimensional stability of the fibers above T_g, which can be indices of orientation, and of the fraction of shrinkable free chains was measured according to ASTM D 2732.[57] Namely, the fibers were annealed in an oven at 70°C in air for 1 h. The shrinkage after annealing was determined by the ratio of the change in length to the original length of the fiber. Also, the shrinkage values of the fibers were measured after immersion in distilled water at 37°C for 4 h to determine the shrinkage of the fibers in the presence of water below T_g under enzymatic degradation conditions.

RESULTS AND DISCUSSION

Dimensional stability

The obtained percentage shrinkage after annealing at 70°C for 1 h in air is shown in Table I. As shown, a large shrinkage was observed for the as-spun fiber F1.0 (48%), whereas the shrinkage values were rather small for the drawn fibers F1.2 and F1.4 (3 and 5%, respectively). Such large shrinkage of the F1.0 fiber should have been due to crystallization of the fiber. This was evidenced by the T_{cc} value of 75.3°C (Table I) and the disappearance of the cold crystallization peak after annealing at 70°C for 1 h in air (DSC curve not shown here). Therefore, for F1.0, the shrinkage value could not be an index of orientation. However, the value difference between F1.2 and F1.4, which showed no cold crystallization during DSC scanning (data shown later), reflected the difference in uniaxial orientation. That is, the degree of uniaxial orientation was higher for F1.4 than for F1.2. On the other hand, the length of the fibers remained unchanged after immersion in distilled water at 37°C for 4 h. This means that the immersion in water had no significant effect on chain orientation or crystallization.

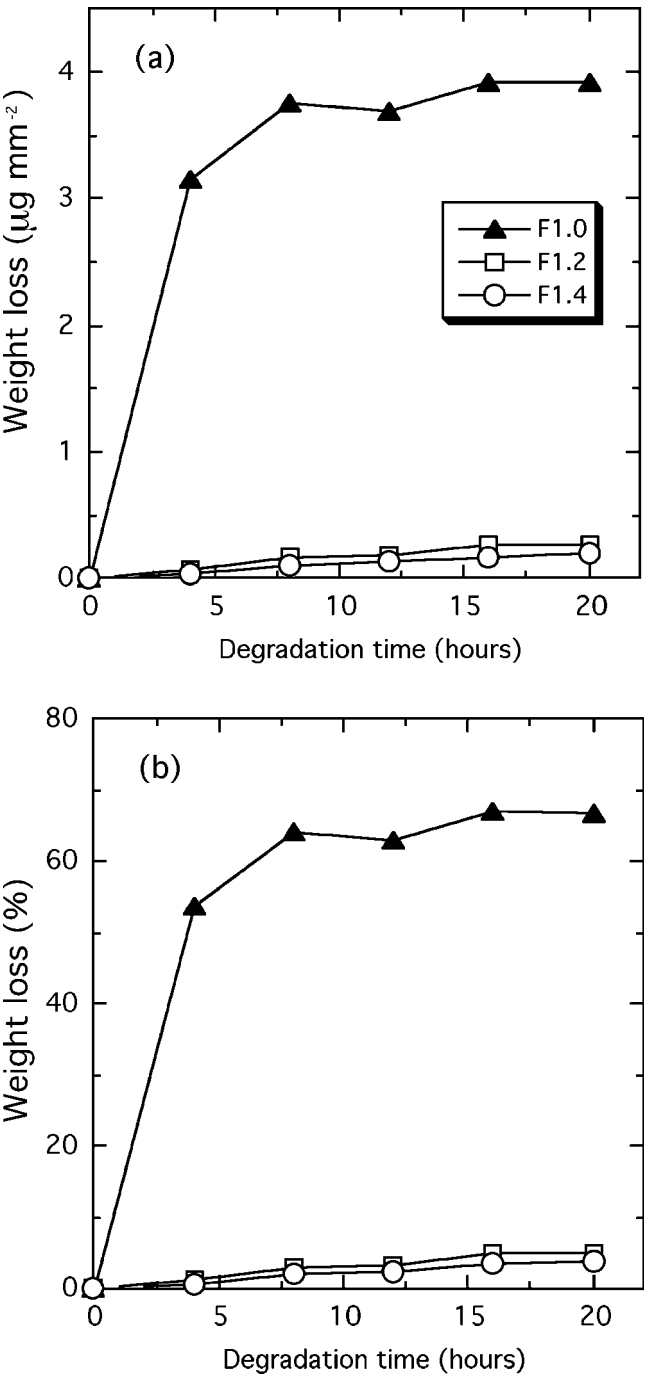

Figure 1 (a) Weight loss per unit surface area and (b) percentage weight loss of the PLLA fibers as a function of enzymatic degradation time.

Weight loss

Figure 1 shows the weight loss of the PLLA fibers with respect to enzymatic degradation time. No significant weight loss was detected for any of the PLLA fibers when they were immersed in the tris-HCl buffer solution without proteinase K for 20 h. This indicated that no significant amount of water-soluble components, such as low-molecular-weight oligomers and monomer, eluted from the PLLA fibers in the absence of proteinase K. Therefore, the weight losses observed for the PLLA fibers, as shown in Figure 1, in the presence of proteinase K could be ascribed to enzyme-catalyzed chain cleavage and to the subsequent elution of water-soluble oligomers and monomer into the surrounding media. Proteinase K is an endoprotease with a fairly broad specificity but a preference for cleavage of peptide bonds C-terminal to aliphatic and aromatic amino acids, especially L-alanine.[65] Probably because of the structure of L-lactic acid analogous to

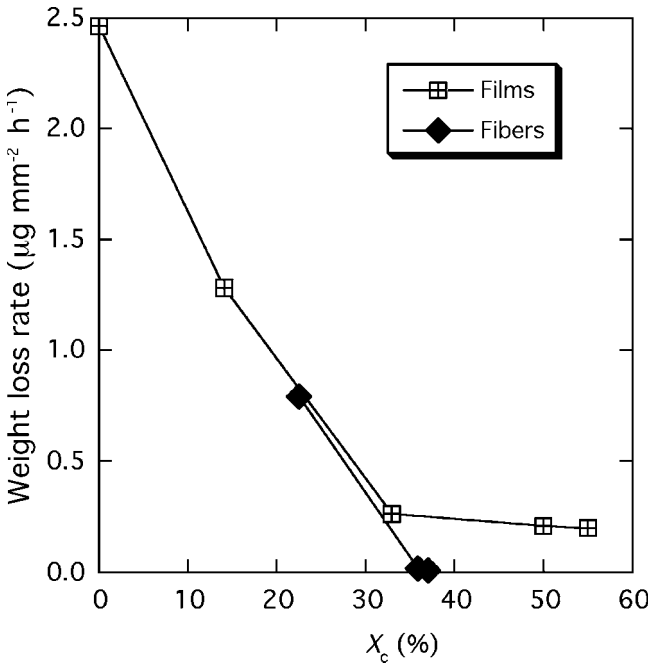

Figure 2 Weight loss rate per unit surface area of the PLLA fibers as a function of X_c, together with reported data for nonoriented PLLA films.[60]

L-alanine, proteinase K could catalyze the hydrolytic degradation of the PLLA chains. Proteinase K could not diffuse into the fibers due to its relatively high molecular weight (28,930 g mol^{-1})[65] and, thereby, catalyzed the hydrolytic degradation of PLLA chains only on the fiber surface.

The as-spun fiber F1.0 showed a rapid increase in weight loss to about 3 μg/mm^2 and 50% in the first 4 h and then leveled off for the periods exceeding 4 h. The weight loss of the drawn fibers F1.2 and F1.4 increased very slowly compared to that of as-spun fiber F1.0. These findings strongly suggest that uniaxial orientation at relatively low draw ratios retarded the enzymatic degradation of the PLLA fibers. To investigate the effects of uniaxial orientation and X_c, the enzymatic degradation rates of the PLLA fibers were estimated from Figure 1, and the obtained weight loss rates and the reported ones for nonoriented PLLA films[60] are plotted in Figure 2 as a function of X_c. For the estimation of weight loss rate of F1.2 and F1.4, all the data for the periods studied here were used, whereas only the data for 0 and 4 h were used for F1.0 due to the rapid saturation of weight loss.

The weight loss rate of nonoriented PLLA films was reported to decrease dramatically for X_c values up to 30% and to decrease very slowly with X_c for X_c values exceeding 30%.[60] This was ascribed to the fact that the main amorphous regions for X_c below and above 30% are free and restricted (or restrained) amorphous regions, respectively. Here, the free amorphous regions are outside the spherulites, and the restricted amorphous region is sandwiched by the two crystalline regions in the spherulites. The chains (especially folding chains) in the restricted amorphous regions are highly resistant to enzymatic degradation. Because of this reason, the enzymatic degradation resistance of restricted amorphous regions are much higher that of the free amorphous regions.

If uniaxial orientation had no significant effects on enzymatic degradation, the weight loss of the PLLA fibers would have shown a similar dependence on X_c with that of nonoriented PLLA films. Although the weight loss rate value of the nonoriented fiber F1.0 was consistent with the weight loss rate of the nonoriented films, the uniaxially oriented fibers F1.2 and F1.4 exhibited much lower weight loss rates than those reported for nonoriented films. It was surprising that such small drawing, to the ratios of 1.2 and 1.4, had dramatic disturbance effects on the enzymatic degradation of the PLLA chains. The difference

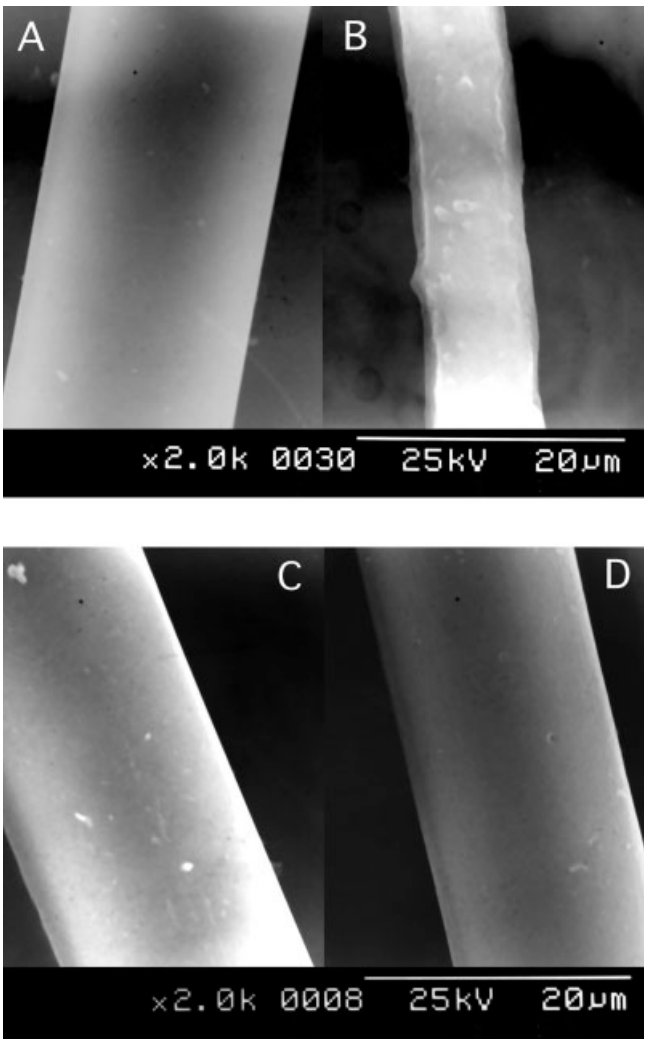

Figure 3 SEM photographs of the PLLA fibers (A and B) F1.0 and (C and D) F1.2: (A and C) before enzymatic degradation and (B and D) after enzymatic degradation for 20 h.

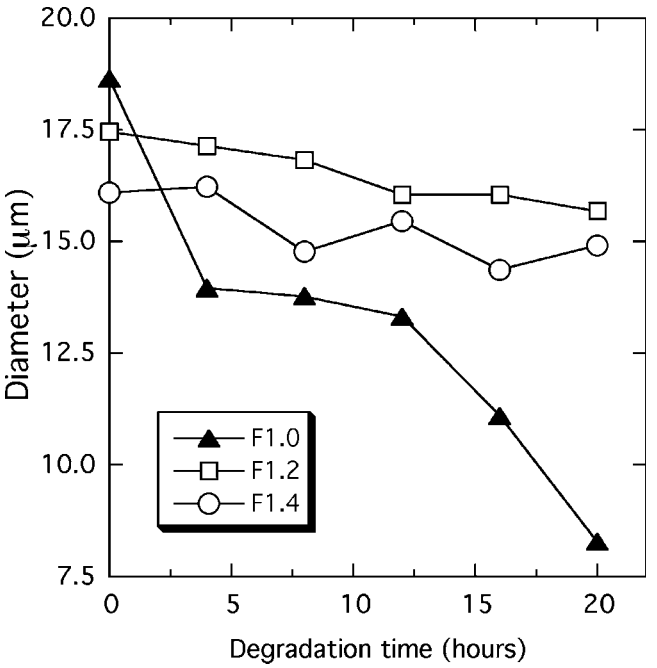

Figure 4 Diameter of the PLLA fibers as a function of enzymatic degradation time.

between the weight loss rates of nonoriented films and the uniaxially drawn fibers was due to the fact that uniaxial orientation disturbed the proteinase K catalyzed enzymatic degradation. This was consistent with the results for poly(ε-caprolactone) fibers in the presence of lipase[66] and also for the uniaxially oriented PLLA films in the presence of proteinase K.[57]

Morphological change

SEM photographs of the fibers before and after enzymatic degradation are shown in Figure 3. The diameter of as-spun fiber F1.0 decreased remarkably after enzymatic degradation, whereas a very small change was observed for the uniaxially drawn fiber F1.2. The surface of F1.0 became uneven after enzymatic degradation. However, structural development, such as periodical transverse crack formation in neutral and alkaline solutions[18,41,42,50] and in composting conditions,[50] was not observed for the PLLA fibers in the presence of proteinase K. The diameter of the fibers with respect to enzymatic degradation time is shown in Figure 4. The diameter of all of the fibers decreased monotonically with enzymatic degradation time without saturation, in contrast to the weight loss saturation of F1.0 for degradation periods exceeding 4 h (Fig. 1). From this finding, the seeming saturation of weight loss shown in Figure 1 was attributable to the decrease of diameter during enzymatic degradation, which was expected to remarkably reduce the weight loss rate at the late stage when a large decease in diameter and, therefore, in surface area per unit mass took place.

Molecular weight distribution

GPC measurements were carried out for the PLLA fibers before and after enzymatic degradation. The molecular distribution curves of all the fibers shown in Figure 5 and their M_n and M_w/M_n (Table I) remained unvaried after enzymatic degradation for 20 h. This was also indicative of the fact that the accumulation of crystalline residues formed as a result of selective cleavage and removal of the amorphous chains did not take place in all of the PLLA fibers, regardless of the draw ratio. The weight loss level was high for the as-spun fiber F1.0 after degradation, and therefore, the fraction of crystalline residues after degradation must have been higher than a traceable level if the crystalline residues were accumulated on the fiber surface. No significant change in the molecular weight distribution curve strongly suggested the facile release of formed crystalline residues, as in the case of biaxially oriented PLLA films in the presence of proteinase K.[56] Such a release may have been caused by the fact that the crystalline regions were oriented with their *c* axis parallel to the machine direction even in the as-spun fiber F1.0. We expect that the crystalline residues were released from the drawn fibers F1.2 and F1.4, as in the case of F1.0. However, we could not confirm the release of crystalline residues due to the low percentage weight loss after degradation (5 and 4%, respectively, for F1.2 and F1.4). At

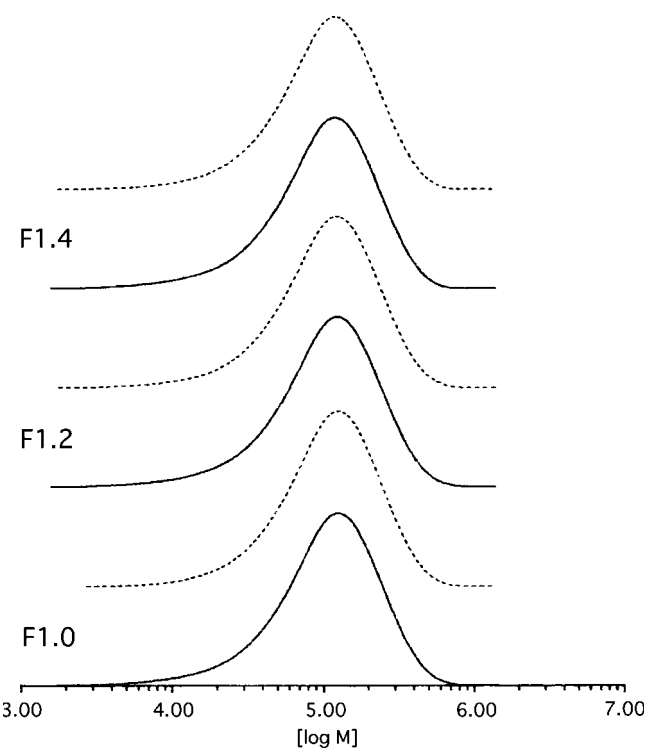

Figure 5 Molecular weight distribution curves of the PLLA (—) fibers before enzymatic degradation and (- - -) after enzymatic degradation for 20 h.

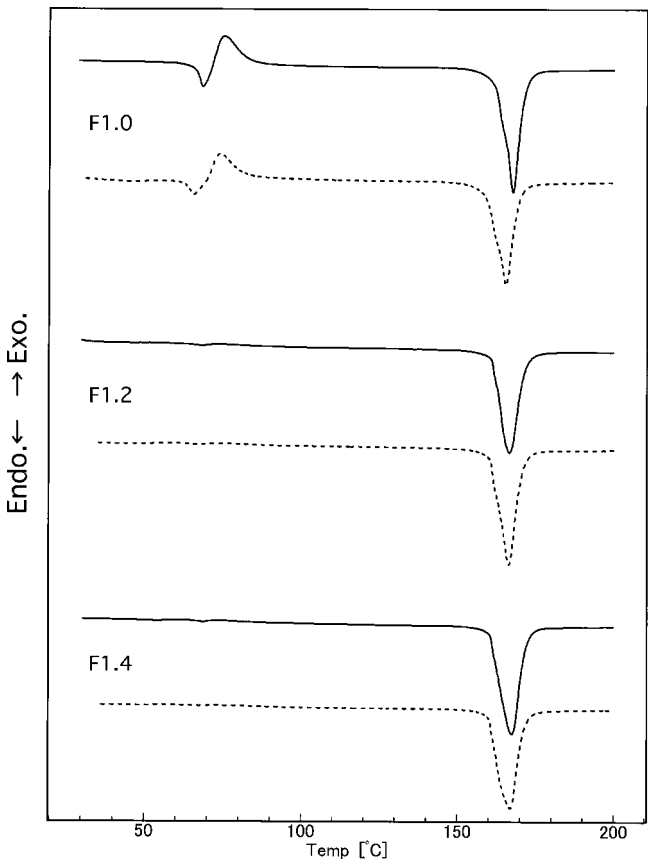

Figure 6 DSC thermograms of the PLLA fibers (—) before enzymatic degradation and (- - -) after enzymatic degradation for 20 h.

such low weight loss values, the accumulated crystalline residues should have been below traceable levels, if accumulated. The results in this section also mean that the enzymatic degradation of PLLA fibers was untraceable by GPC.

Changes in higher ordered structures

The DSC thermograms of the fibers before and after enzymatic degradation are shown in Figure 6. Glass transition, cold crystallization (only for F1.0), and melting peaks were observed for the PLLA fibers before and after enzymatic degradation around 60, 75, and 165°C, respectively. The shape and positions of these peaks remained practically unchanged after enzymatic degradation, and the changes in the thermal properties obtained from the DSC thermograms were negligibly small. This was confirmed by no significant changes in X_c, T_g, T_{cc}, and T_m values (Table I). These findings reflect no significant changes in the highly ordered structures in the fibers and no significant accumulation of the crystalline residues during enzymatic degradation. The results in this section also reveal that DSC could not monitor the enzymatic degradation of the PLLA fibers.

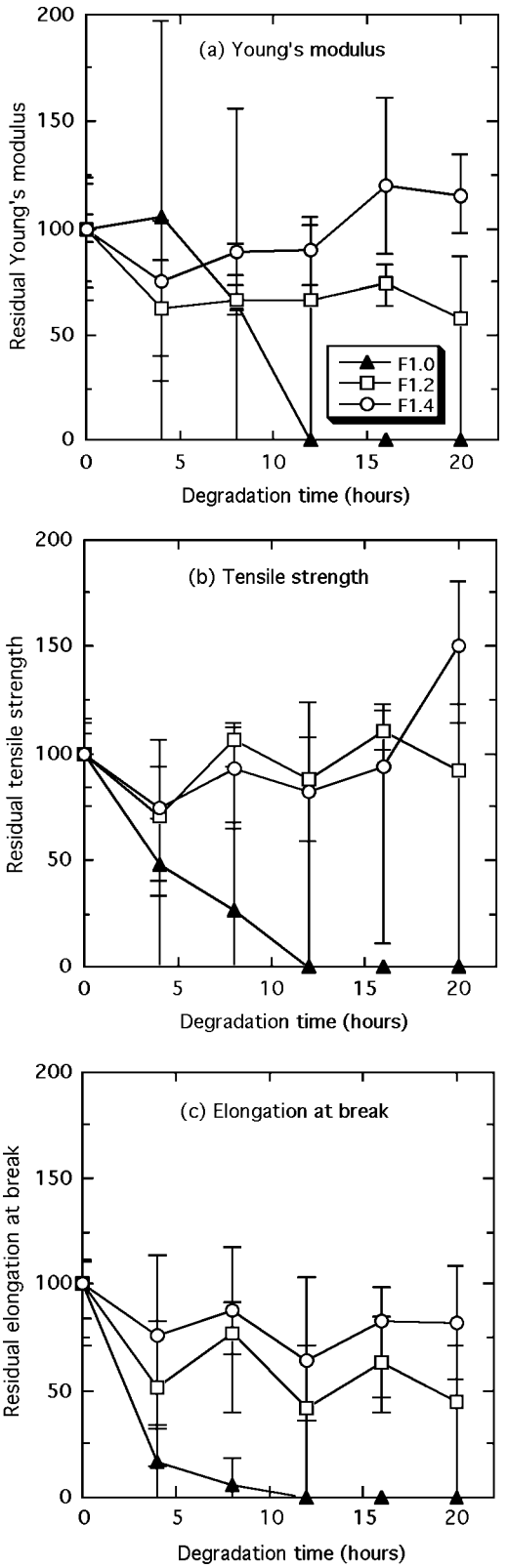

Figure 7 (a) Young's modulus, (b) tensile strength, and (c) elongation at break of the PLLA fibers as a function of enzymatic degradation time.

Changes in the mechanical properties

Figure 7 shows the mechanical properties of the fibers with respect to enzymatic degradation time. As presumed from the aforementioned results, the mechanical properties of the uniaxially drawn fibers F1.2 and F1.4 remained unvaried for the degradation period studied here, whereas those of the as-spun fiber F1.0 decreased rapidly and reached zero at 12 h. This confirms that the uniaxial orientation elevated the enzymatic degradation resistance of the PLLA fibers. The dramatic decrease in the tensile properties of F1.0 was attributed to the uneven surface, as shown by SEM observation.

CONCLUSIONS

From the aforementioned results, the following conclusions were derived for the effects of uniaxial orientation on the enzymatic degradation of PLLA fibers:

1. It was surprising that uniaxial orientation at relatively low draw ratios dramatically disturbed the proteinase K catalyzed enzymatic degradation of the PLLA fibers. This was because the enzyme could not attach to the extended (strained) chains in the amorphous regions of the uniaxially oriented PLLA fibers or could not catalyze the cleavage of the strained chains.
2. The accumulation of crystalline residues formed as a result of selective cleavage, and removal of the amorphous chains was not observed even for the as-spun PLLA fibers. This indicated the facile release of formed crystalline residues from the surface of the as-spun PLLA fibers during enzymatic degradation. Such a release may be explained by the fact that the crystalline regions of the as-spun PLLA fibers were oriented with their *c* axis parallel to the machine direction, as reported for biaxially oriented PLLA films.
3. Gravimetry, SEM, and tensile testing traced the enzymatic degradation of the PLLA fibers, whereas the enzymatic degradation of the PLLA fibers was untraceable by GPC and DSC.

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