Enzymatic, Alkaline, and Autocatalytic Degradation of Poly(L-lactic acid): Effects of Biaxial Orientation

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The hydrolytic degradation of biaxially oriented and de-oriented (melt-crystallized) poly(L-lactic acid) (PLLA) films was investigated in Tris-HCl-buffered solution (pH 8.6) with proteinase K, alkaline solution, and phosphate-buffered solution (pH 7.4) by the use of gravimetry, gel permeation chromatography, differential scanning calorimetry, and scanning electron microscopy. Biaxial orientation disturbed the proteinase K-catalyzed enzymatic degradation of PLLA films and the effects of biaxial orientation overcame those of crystallinity. The former may be due to the fact the enzyme cannot attach to the extended (strained) chains in the amorphous regions of the biaxially oriented PLLA film or cannot catalyze the cleavage of the strained chains. Another probable cause is that the enzyme can act only at the film surface of the biaxially oriented PLLA film, in marked contrast with the case of the de-oriented PLLA films where enzymatic degradation can proceed beneath the spherulitic crystalline residues. The effects of biaxial orientation on the alkaline and autocatalytic degradation of the PLLA films were insignificant for the periods studied here. The crystallinity rather than the biaxial orientation seems to determine the alkaline and autocatalytic degradation rates of the PLLA films. The accumulation of crystalline residues formed as a result of selective cleavage and removal of the amorphous chains was observed for the de-oriented PLLA films, but not for the biaxially oriented PLLA film, when degraded in the presence of proteinase K. This means the facile release of formed crystalline residues from the surface of the biaxially oriented PLLA film during enzymatic degradation, due to the fact that the crystalline regions of the biaxially oriented PLLA film were oriented with their *c* axis parallel to the film surface.

Introduction

Recently, a large number of articles have appeared on the physical properties, crystallization, and degradation of poly(L-lactic acid) (PLLA), because PLLA is attractive in terms of the producibility from renewable resources such as starch and the functionality of "hydrolytic degradability (hydrolyzability)" in the presence of water.[1−12] These features as well as non- or very low toxicity of PLLA are useful for environmental and medical applications.

Among numerous highly ordered structural parameters, the effects of crystallinity (X_c) on the autocatalytic,[13−28] alkaline,[28−31] and enzymatic[28,31−38] degradation of PLLA have been intensively studied. It was found that the alkaline and enzymatic (proteinase K-catalyzed) degradability of PLLA becomes lower with X_c, whereas the autocatalytic degradability of PLLA in neutral media such as phosphate-buffered solution increases with X_c. The latter unexpected result can be explained by the enhanced hydrolytic degradation of the amorphous regions between the crystalline regions due to the following three factors: (1) the increased hydrophilicity by increases in the densities of hydrophilic terminal groups (hydroxyl and carboxyl groups), (2) the elevated autocatalytic effects through an increase in the density of catalytic carboxyl groups, and (3) the increased disorder in chain packing in the amorphous regions by increases in the densities of terminal groups. These three factors extremely enhance the hydrolytic degradability of chains in the amorphous regions between the crystalline regions, resulting in rapid overall degradation.[20,21]

The hydrolytically degradable PLLA chains in the amorphous regions between the crystalline regions are different in autocatalytic and alkaline degradation and in enzymatic degradation. Namely, in the former all kinds of the chains (tie chains, folding chains, and the chains with a free end) can be degraded, whereas in the latter folding chains are hydrolysis-resistant but tie chains and the chains with a free end are degradable.[11,20,21,30] Because folding chains are hydrolysis-resistant, the proteinase K-catalyzed degradation rate of the PLLA chains in the amorphous regions between the crystalline regions ("restricted" amorphous regions) is much lower than that in the amorphous chains outside the spherulites ("free" amorphous regions).[36,37] Furthermore, Iwata and Doi[39] and Kikkawa et al.[40] investigated in detail the mechanisms of proteinase K-catalyzed enzymatic degradation of PLLA single crystals and melt-crystallized thin films, respectively.

On the other hand, the effects of orientation as another highly ordered structural parameter on the autocatalytic degradation of PLLA was originally reported by Jamshidi et al. and they found that uniaxial orientation of PLLA fibers induced the retardation of hydrolytic degradation in phosphate-buffered solution.[41] Similar results were reported by Lee et al.[42] and Cai et al.[34] for PLLA specimens, when degraded in phosphate-buffered solution and in Tris-HCl-buffered solution with proteinase K, respectively. In contrast, Burg et al. observed a higher flexural modulus of poly(L-lactide-*co*-D-lactide)(75/25) for uniaxially oriented films than that of nonoriented films after

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degradation in phosphate-buffered solution.[43] However, as far as we are aware, there has been no report for the effects of biaxial orientation on the hydrolytic degradation of PLLA in various types of media, despite the fact that the biaxially oriented PLLA films are used for a wide variety of applications which require the functionality of "hydrolytic degradability".

The objectives of the present study were to investigate the effects of biaxial orientation on the hydrolytic degradation (enzymatic, alkaline, and autocatalytic degradation) of PLLA and to compare the results obtained for different degradation media with each other. For these purposes, we have obtained a biaxially oriented PLLA film and prepared de-oriented PLLA films by the crystallization of the oriented PLLA film from the melt. To remove the effects of X_c, crystalline thickness, and molecular weight of PLLA on its hydrolytic degradation, the de-oriented (melt-crystallized) PLLA films were prepared to have similar X_c, melting temperature (T_m), and molecular weight values with those of the biaxially oriented PLLA film. The hydrolytic degradation behavior of these specimens were carried out in three types of degradation media: Tris-HCl-buffered solution with proteinase K, alkaline solution, and phosphate-buffered solution. The hydrolyzed specimens were analyzed with gravimetry, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM).

Experimental Section

Materials. A biaxially oriented PLLA film (abbreviated as PLLA-O film) was kindly supplied by Shimadzu Co. (Kyoto, Japan). De-oriented PLLA films were prepared by the following procedure. Each of the PLLA-O films was placed between two Teflon sheets and then sealed in a glass tube under a reduced pressure. The sealed films were melted at 200 °C for 5 min and then crystallized at 100 and 110 °C for 10 hours, followed by quenching at 0 °C to stop further crystallization.[20,21,36,37] The de-oriented PLLA films prepared by crystallization at 100 and 110 °C are abbreviated as PLLA-C(100) and PLLA-C(110) films, respectively.

Tris(hydroxymethyl)aminomethane (the specially prepared reagent, nuclease and proteinase tested), sodium azide (the guaranteed grade), 0.1 M NaOH solution, 0.1 M HCl solution, and distilled water (the HPLC grade) were purchased from Nacalai Tesque Inc. (Kyoto, Japan) and used without further purification. The distilled water was used for the preparation of phosphate-buffered and Tris-HCl-buffered solutions and for the washing of degraded films. Proteinase K (lyophilized powder, 80% protein) was purchased from Sigma-Aldrich Co. (St. Louis, MO) and used as received.

Hydrolytic Degradation. The autocatalytic degradation of each of the films (PLLA-O: 30 mm × 20 mm × 20 μm, PLLA-C: 25 mm × 10 mm × 25 μm) was performed in a vial filled with 400 mL of phosphate-buffered solution (pH 7.4) containing 80 mg of sodium azide at 37 °C in an incubator for the periods up to 6 months.[20,21] The phosphate-buffered solution was replaced once a month. The alkaline degradation of each of the films was carried out in a vial filled with 2.5 mL/(100 mm^2 of surface area of a film) of 0.1 M NaOH solution at 37 °C in a rotary shaker for the periods up to 20 h (25 h for SEM observation).[30,31] The enzymatic degradation of the films was performed according to the procedure reported by Reeve et al.[32] Namely, each of the films was placed in a vial filled with 2.5 mL/(100 mm^2 of surface area of a film) of Tris-HCl buffer solution (pH 8.6) containing 0.2 mg/mL of proteinase K and 0.2 mg/mL of sodium azide. The enzymatic degradation of the films was carried out at 37 °C in a rotary shaker for the periods up to 30 h. The pH of the solution remained in the range between 8.6 and 8.0 in 30 h, where the enzyme activity was reported to be practically constant.[32]

The degraded films were washed thoroughly with the distilled water at 4 °C to stop further degradation and then dried under a reduced pressure for at least 2 weeks for weight loss and thermal measurements. The experimental weight loss values for enzymatic degradation represent averages of measurements from the three replicate specimens.

Measurements. Without catalytic substances as in a phosphate-buffered solution, PLLA specimens are degraded via a bulk erosion mechanism. In contrast, in the presence of alkalis and proteinase K, it is known that PLLA specimens are degraded via a surface erosion mechanism.[11] Therefore, the percentage weight loss [W_{loss} (%)] and the weight loss per unit surface area of the degraded films [W_{loss} (μg mm^{-2})] were calculated by the following equations using the weights of the film before and after degradation (W_{before} and W_{after}, respectively) and the film surface area before degradation (S_{before}), depending on the degradation mechanisms:

$$W_{loss}\,(\%) = (W_{before} - W_{after})/W_{before} \times 100 \quad \text{(autocatalytic degradation)} \quad (1)$$

$$W_{loss}\,(\mu g\ mm^{-2}) = (W_{before} - W_{after})/S_{before} \quad \text{(alkaline and enzymatic degradation)} \quad (2)$$

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

The glass transition and melting temperatures (T_g and T_m, respectively) and enthalpy of melting (ΔH_m) of the films were determined by a Shimadzu (Kyoto, Japan) DSC-50 differential scanning calorimeter. The films (sample weight of ca. 3 mg) were heated at a rate of 10 °C min^{-1} from room temperature to 200 °C under a nitrogen gas flow at a rate of 50 mL min^{-1}. T_g, T_m, and ΔH_m were calibrated using benzophenone, indium, and tin as standards. The X_c of the films was evaluated according to the following equation:[25,27]

$$X_c\,(\%) = 100\ \Delta H_m/135 \quad (3)$$

where 135 J g^{-1} is ΔH_m of PLLA crystal having an infinite size reported by Miyata and Masuko.[44]

The degrees of orientation of the PLLA films before hydrolytic degradation were evaluated by X-ray diffractometry according to the simplified method developed by Go and Kubo[45,46] using diffraction peak of a (200) plane of α-modification ($2\theta = 16.8°$) with rotationable specimen holder. The X-ray diffratometry was carried out with Rigaku (Tokyo, Japan) RAD-rB diffractometer equipped with a Cu Kα source at room temperature. The characteristics and properties of the PLLA films before and after the degradation are summarized in Table 1. As seen in Table 1, the de-oriented PLLA-C films have orientation degrees much lower than that of the biaxially oriented PLLA-O film. The morphology of the films was studied with a Hitachi (Tokyo, Japan) SEM (X-650). The films for SEM observation were coated with Pt to a thickness of about 20 nm.

Results and Discussion

Weight Loss. Figure 1 shows the weight loss of the PLLA films with respect to hydrolytic degradation time in different media. No significant weight loss was detected for all PLLA films when they were immersed in distillated water without NaOH and in the Tris-HCl-buffered solution without proteinase K for 25 and 30 h, respectively. This indicates that no significant amount of water-soluble components such as low-molecular-weight oligomers and monomer eluted from the PLLA films in the absence of NaOH and proteinase K. Therefore, the weight losses observed for the PLLA films as shown in Figure 1, panels

Table 1. Characteristics and Properties of PLLA Films before and after Hydrolytic Degradation

code	degradation type	time	degree of orientaion[a] (%)	M_n (g mol^{-1})	M_w/M_n	X_c[b] (%)	T_g[c] (°C)	T_m[d] (°C)
PLLA-O		0	92	1.19×10^5	1.60	20.2	66.3	152.0
	autocatalytic	6 months		0.73×10^5	1.63	23.8	66.8	151.6
	alkaline	20 hours		1.16×10^5	1.66	18.8	64.1	151.4
	enzymatic	30 hours		0.94×10^5	2.01	19.7	67.8	151.9
PLLA-C(100)		0	76	1.01×10^5	1.72	27.8	55.7	149.0
	autocatalytic	6 months		0.73×10^5	1.62	30.2	63.6	149.4
	alkaline	20 hours		1.18×10^5	1.63	31.0	60.6	149.1
	enzymatic	30 hours		0.29×10^5	5.42	34.9	61.5	148.4
PLLA-C(110)		0	67	1.01×10^5	1.63	29.2	54.6	152.0
	autocatalytic	6 months		0.81×10^5	1.54	30.1	63.6	152.2
	alkaline	20 hours		1.21×10^5	1.59	31.8	61.0	152.0
	enzymatic	30 hours		0.22×10^5	6.49	38.5	61.0	151.6

[a] Orientation index values were estimated by X-ray diffractometry. [b] Crystallinity. [c] Glass transition temperature. [d] Melting temperature.

b and c, in the presence of NaOH and proteinase K can be ascribed to the alkaline chain cleavage and enzyme-catalyzed chain cleavage, respectively, and to the subsequent elution of water-soluble oligomers and monomer into the surrounding media.

As seen in Figure 1, significant induction periods until the onset of weight loss were observed for autocatalytic degradation of all PLLA films, whereas weight loss of all PLLA films occurred without any induction periods for alkaline and enzymatic degradation. The former and latter weight loss behavior are typical when the hydrolytic degradation proceeds via bulk and surface erosion mechanisms, respectively.[11] Proteinase K is an endoprotease having a fairly broad specificity but a preference for cleavage of peptide bonds C-terminal to aliphatic and aromatic amino acids, especially L-alanine.[47] Probably due to the structure of L-lactic acid analogous to L-alanine, proteinase K can catalyze the hydrolytic degradation of PLLA chains. Proteinase K cannot diffuse into the film because of its relatively high molecular weight (28 930 g mol^{-1})[47] and thereby catalyzes the hydrolytic degradation of PLLA chains only at the film surface.

In the phosphate-buffered solution, the hydrolytic degradation of the PLLA-O film (initial $X_c = 20.2\%$) [Figure 1a] was slightly disturbed compared to that of PLLA-C films [initial $X_c = 27.8\%$ and 29.2% for PLLA-C(100) and PLLA-C(110) films, respectively]. Reversely, in the alkaline solution, the hydrolytic degradation of the PLLA-O film [Figure 1b] was enhanced compared to that of PLLA-C films. These tendencies can be explained by the X_c effects rather than the effects of degree of orientation on the hydrolytic degradation of PLLA specimens. That is, the increase in X_c enhances and disturbs the hydrolytic degradation of the PLLA specimens in the phosphate-buffered solution and the alkaline solution, respectively, as stated in the Introduction. However, in the case of autocatalytic degradation, the weight loss values of PLLA specimens for the period studied here were too small to conclude the effects of X_c and biaxial orientation. In alkaline degradation, the actual surface areas of PLLA-C films seem to be larger than that of PLLA-O film, as shown in the Morphological Change section. This effect will elevate the seeming alkaline degradation rates of PLLA-C films over that of PLLA-O film. However, such effect was not so dramatic to overwhelm the effects of X_c.

In enzymatic degradation [Figure 1c], the de-oriented PLLA-C films with higher initial X_c values were expected to have lower degradation rates than that of the biaxially oriented PLLA-O film with a lower X_c value.[11,32,36] However, the enzymatic weight loss rates of PLLA-C films were higher than that of the PLLA-O film. This reflects the fact that biaxial orientation disturbs the enzymatic degradation of PLLA films and the effects of biaxial orientation overcome those of X_c. It is probable that the enzyme cannot attach to the extended (strained) chains in the amorphous regions of the biaxially oriented PLLA film or cannot catalyze the cleavage of the strained chains. The disturbed enzymatic degradation of PLLA-O film compared to that of PLLA-C films agrees well with the results reported for uniaxially oriented and de-oriented PLLA films.[34]

Molecular Weight Change. In the phosphate-buffered solution, with degradation time, the molecular weight distribution curves of all PLLA films shifted to a lower molecular weight as a whole, without the formation of any low-molecular-weight specific peaks for the crystalline residues (data not shown here). These are typical molecular weight changes for a bulk erosion mechanism at an early stage of degradation. In the alkaline solution, with degradation time, neither significant shape and position changes in GPC curves nor low-molecular-weight specific peaks were observed for all PLLA films (data not shown here). These are typical changes for a surface erosion of PLLA films at an early stage. As reported earlier,[30] for the PLLA films prepared by crystallization at temperatures above 120 °C, the accumulation of crystalline residues was observed at a late stage of alkaline degradation. However, for the PLLA films prepared by crystallization at temperatures lower than 120 °C as in the present study, no accumulation of crystalline residues was observed during alkaline degradation even at a late stage. It is probable that the small-sized crystalline residues formed from the PLLA films prepared by the crystallization at lower temperatures may be released readily from the film surface or promptly degraded to water-soluble oligomers and monomer.

Figure 2 gives the GPC curves of the PLLA films before and after enzymatic degradation. For the de-oriented PLLA-C films, specific low-molecular-weight multiple peaks were observed at the molecular weight in the range of $2 \times 10^3 - 3 \times 10^4$ g mol^{-1}, regardless of the crystallization temperatures of the films. This reflects the fact that the crystalline residues were accumulated during enzymatic degradation, in marked contrast with the results for alkaline degradation. The multiple low-molecular-weight specific peaks correspond to one, two, and 3-folds of PLLA chain in the crystalline regions. These specific peaks were formed as a result of a much higher degradation-resistance of the folding chains in the presence of proteinase K than that of the tie chains and the chains with a free end. The molecular weight of the lowest specific peak was higher for PLLA-C(110) film prepared at 110 °C than for PLLA-C(100)

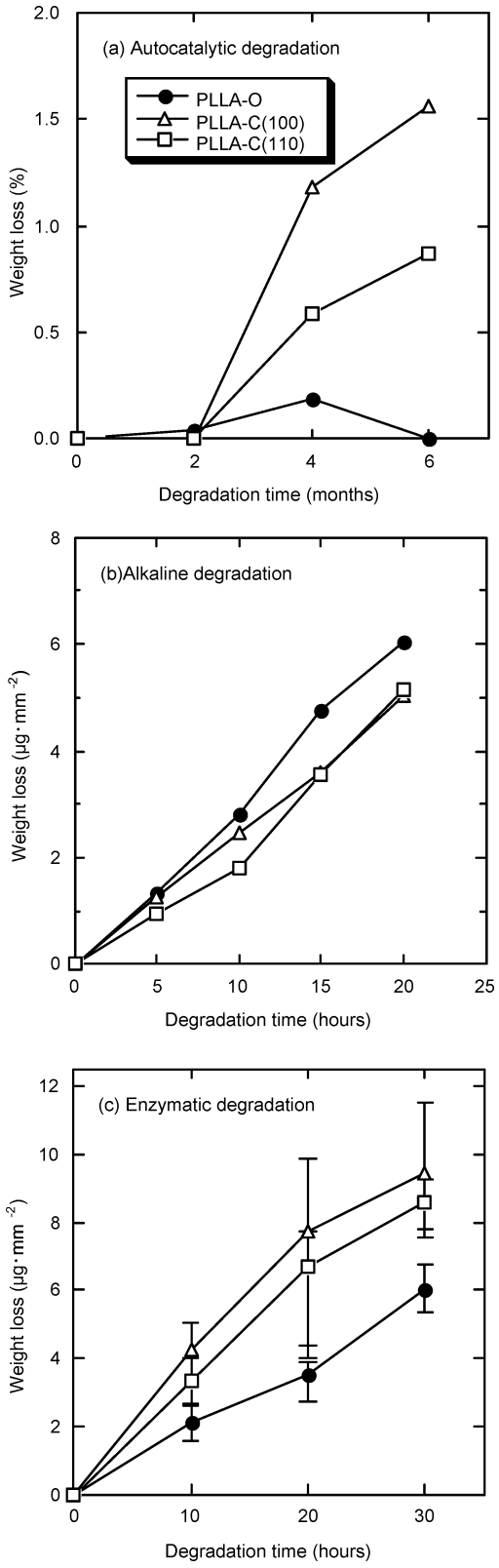

Figure 1. Weight loss of PLLA-O, PLLA-C(100), and PLLA-C(110) films degraded in phosphate-buffered solution (a), alkaline solution (b), and Tris-HCl-buffered solution with proteinase K (c) as a function of degradation time.

film prepared at 100 °C. This reflects the fact that the crystalline thickness of PLLA films increases with crystallization temperature. The similar results were reported for the hydrolytic degradation of crystallized PLLA specimens in a phosphate-buffered solution at 37 °C for the periods up to 3 years[21] or at

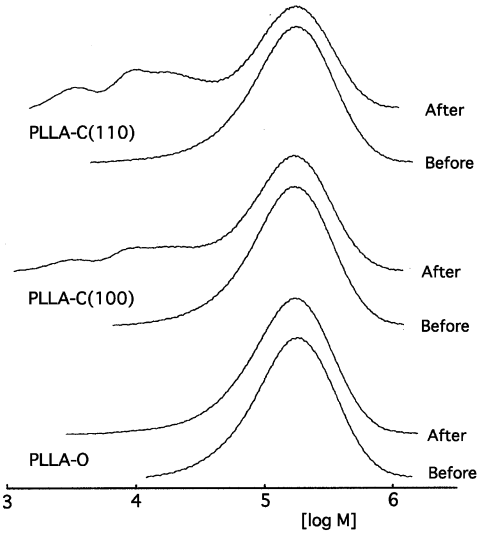

Figure 2. GPC curves of PLLA-O, PLLA-C(100), and PLLA-C(110) films before degradation and after enzymatic degradation for 30 h.

97 °C for the periods up to 40 h,[25] in an alkaline solution at 37 °C for the periods up to 150 days,[30] and in a Tris-HCl-buffered solution with proteinase K at 37 °C for the periods up to 40 h.[37]

It is interesting to note that the biaxially oriented PLLA-O film showed no such specific peaks, despite the fact that the PLLA-O film had a crystalline thickness similar to that of the de-oriented PLLA-C(110) film as expected from the identical T_m values (152.0 °C). This means that the crystalline residues of the PLLA-O film were released from the film surface during enzymatic degradation, leaving a negligibly small amount of the crystalline residues on the film surface. Facile release of the crystalline residues may have arisen from the fact that the crystalline regions of the PLLA-O film were oriented with their *c*-axis parallel to the film surface, in marked contrast with the random orientation of the crystalline regions in PLLA-C films. In the case of enzymatic degradation, there is no probability that the crystalline residues are rapidly degraded to water-soluble oligomers and monomer, because proteinase K cannot catalyze hydrolytic degradation of the chains in the crystalline regions.[28]

Figures 3 and 4 show the M_n and M_w/M_n of the PLLA films, respectively, with respect to hydrolytic degradation time. As anticipated from the small and insignificant changes in GPC curves, for autocatalytic degradation, the M_n of all PLLA films decreased very slowly at similar rates [Figure 3a], whereas for alkaline degradation, the M_n of all PLLA films showed no significant changes [Figure 3b]. As also expected from the small and insignificant changes in GPC curves for autocatalytic and alkaline degradation, respectively, the M_w/M_n of all PLLA films remained unchanged for the period studied here [Figure 4, panels a and b]. In contrast, for enzymatic degradation, PLLA-C films showed rapid decrease in M_n and increase in M_w/M_n at degradation periods of 30 and 20 h respectively for PLLA-C(100) and C(110) films [Figure 4c]. This can be explained by the accumulation of crystalline residues on the film surface during enzymatic degradation. On the other hand, no such changes in M_n and M_w/M_n were observed for PLLA-O film [Figure 4c]. These findings for enzymatic degradation support the fact that most of the crystalline residues of PLLA-O film were released from the film surface.

Changes in Highly-Ordered Structure. The T_g, T_m, and X_c of the PLLA films before and after the degradation are given in Table 1. The T_g of all PLLA films increased after three types

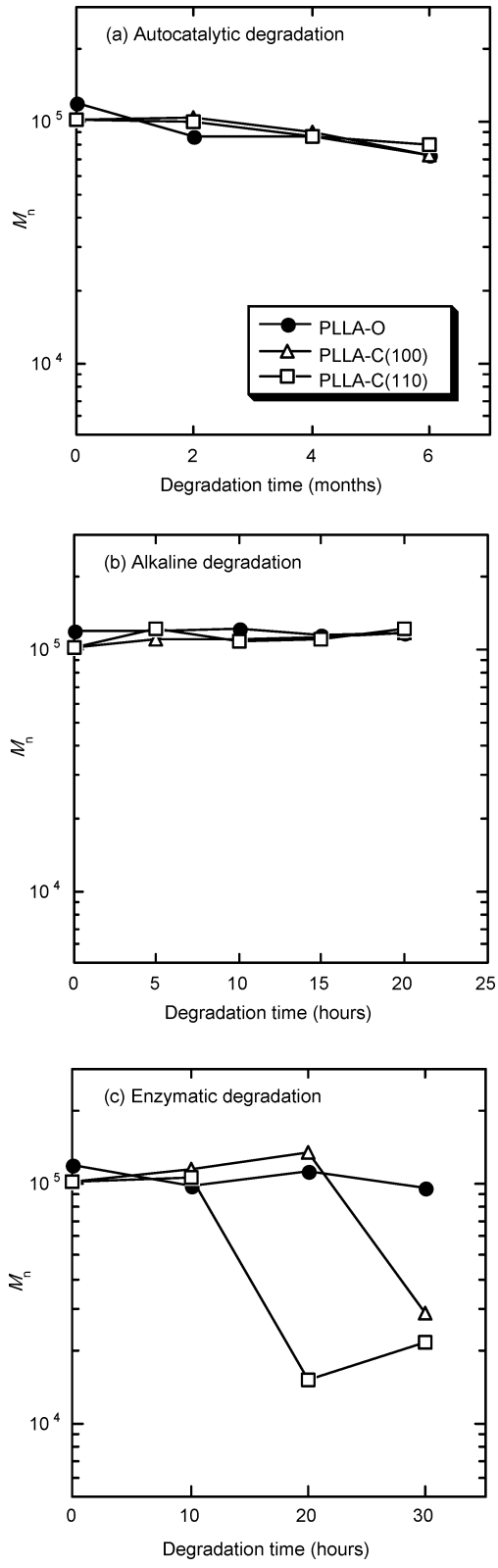

Figure 3. Number-average molecular weight (M_n) of PLLA-O, PLLA-C(100), and PLLA-C(110) films degraded in phosphate-buffered solution (a), alkaline solution (b), and Tris-HCl-buffered solution with proteinase K (c) as a function of degradation time.

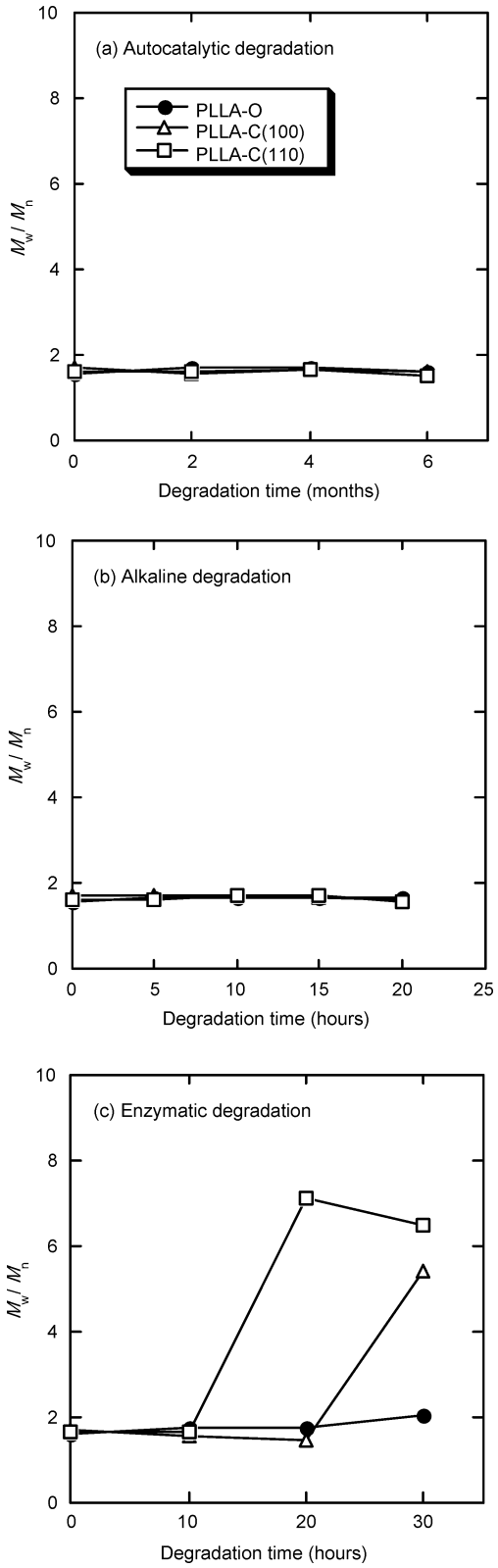

Figure 4. Weight-average molecular weight (M_w)/number-average molecular weight (M_n) of PLLA-O, PLLA-C(100), and PLLA-C(110) films degraded in phosphate-buffered solution (a), alkaline solution (b), and Tris-HCl-buffered solution with proteinase K (c) as a function of degradation time.

of degradation, except for the PLLA-O film after alkaline degradation. This reflects the fact that chain packing in the amorphous regions was stabilized by the hydrolytic degradation or the annealing at a slightly elevated temperature in the presence of water as a plasticizer. On the other hand, the T_m of all PLLA films showed very small changes after three types of degradation. This means that the fractions of the crystalline residues having a lower thickness or a different surface structure were very low in the PLLA films after degradation. However, we cannot explain why T_g of the PLLA-O film decreased after alkaline degradation. On the other hand, as illustrated in Figure

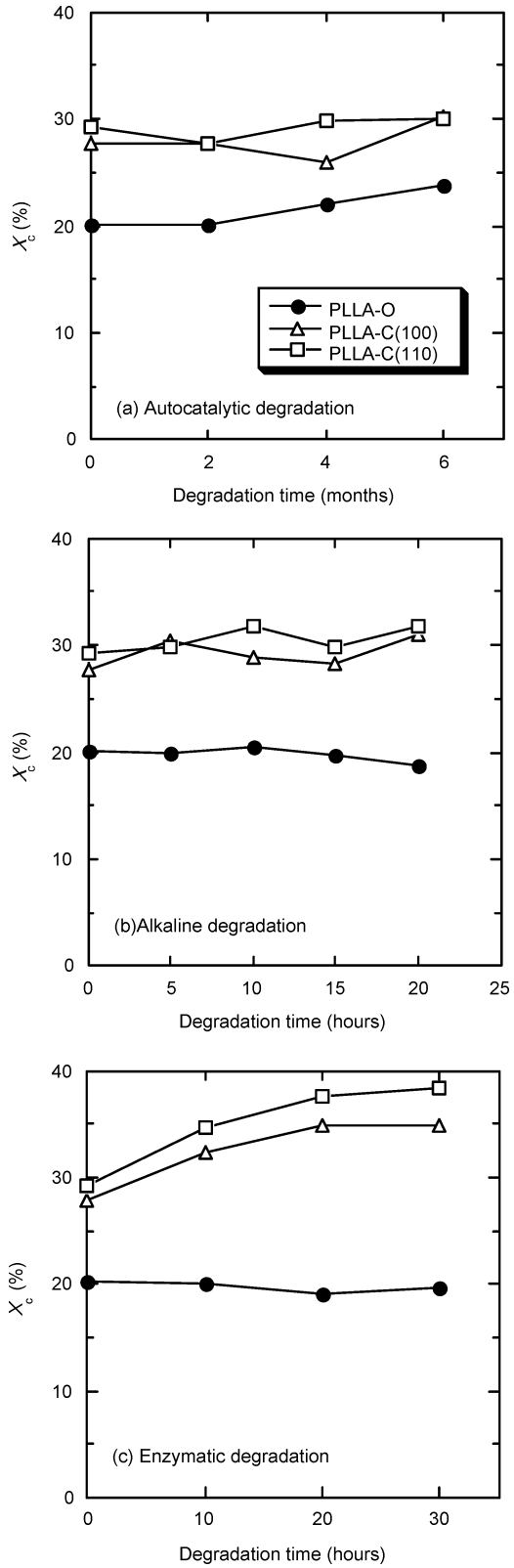

Figure 5. Crystallinity (X_c) of PLLA-O, PLLA-C(100), and PLLA-C(110) films degraded in phosphate-buffered solution (a), alkaline solution (b), and Tris-HCl-buffered solution with proteinase K (c) as a function of degradation time.

5, insignificant or very small changes in X_c took place for all PLLA films degraded in the phosphate-buffered and alkaline solutions and for the PLLA-O films degraded in the Tris-HCl-buffered solution with proteinase K. Probably, this is ascribed to fact that the amount of crystalline residues accumulated on the film surface was negligibly low and the crystallization rate was very low during degradation. The X_c of PLLA-C films in the Tris-HCl-buffered solution with proteinase K became higher with degradation time. This validates the fact that the crystalline residues in the PLLA-C films were accumulated during enzymatic degradation. The increases in X_c [Figure 5c] and M_w/M_n [Figure 4c] and the decrease in M_n [Figure 3c] of PLLA-C(110) film occurred rapidly compared to those of PLLA-C(100) film. This result reveals that the accumulation rate of crystalline residues was higher for the de-oriented PLLA-C film crystallized at the higher temperature, despite the fact that the enzymatic degradation rate was lower for the de-oriented PLLA film crystallized at the higher temperature [Figure 1c].

Morphological Change. Figures 6 and 7 respectively show the SEM photographs of the PLLA-C(100) and PLLA-O films before degradation and after alkaline degradation or enzymatic degradation. The SEM photos for the PLLA films after autocatalytic degradation were not shown here, because no significant changes were observed after degradation. The PLLA-C(100) film before degradation had an uneven surface, probably due to the roughness of the Teflon sheets used for thermal treatments. After alkaline and enzymatic degradation, a spherulitic structure was noticed on the surface of PLLA-C(100) films, as reported earlier.[30,37,48] However, the outline of the spherulitic structure is clearer in the present study. It should be noted that despite the formation of the spherulitic structure in the alkaline solution, the fraction of accumulated crystalline residues was negligibly small, as traced by GPC and DSC. On the other hand, the surface of the PLLA-O film was smooth before degradation. Interestingly, most of the surface of PLLA-O films remained smooth after alkaline and enzymatic degradation, although small holes were observed on the surface of the films. This result confirms the fact that the crystalline residues were released readily from the surface of the PLLA-O film during enzymatic degradation, leaving a negligibly small amount of the crystalline residues. The surface structure of the PLLA-C film after enzymatic degradation [Figure 6c] strongly suggests that the enzyme can diffuse inside the films through the spherulitic crystalline residues and catalyze the hydrolytic degradation of amorphous chains beneath the spherulitic crystalline residues. In contrast, in the enzymatic degradation of the PLLA-O film, the enzyme only acts at the film surface. In addition to the higher hydrolysis-resistance of the oriented (strained) chains in PLLA-O film in enzymatic degradation, this may be another cause for the lower enzymatic degradation rate of PLLA-O film.

Conclusions

From the aforementioned results the following conclusions can be derived for the effects of biaxial orientation on the enzymatic, alkaline, and autocatalytic degradation of PLLA films:

(1) Biaxial orientation disturbed the proteinase K-catalyzed enzymatic degradation of PLLA films and the effects of biaxial orientation overcame those of crystallinity. The former may be due to the fact the enzyme cannot attach to the extended (strained) chains in the amorphous regions of the biaxially oriented PLLA-O film or cannot catalyze the cleavage of the strained chains. Another probable cause is that the enzyme can act only at the film surface of PLLA-O film, in marked contrast with the case of de-oriented PLLA-C films where enzymatic degradation can proceed beneath the spherulitic crystalline residues.

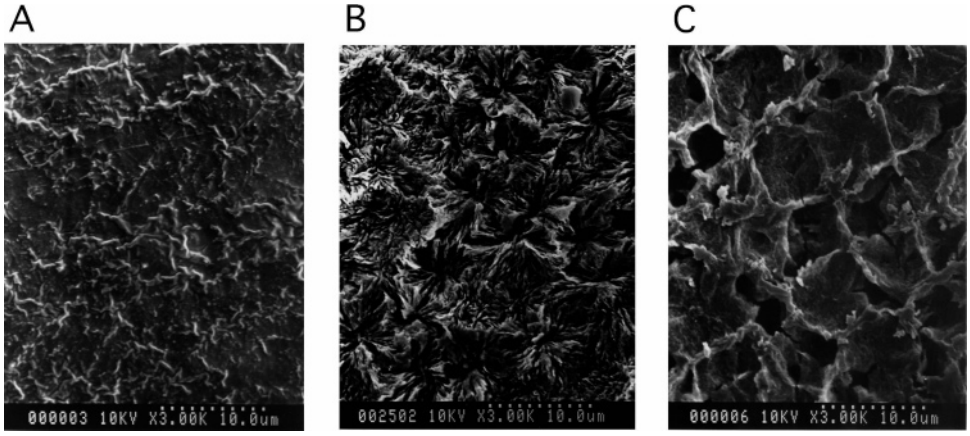

Figure 6. SEM photographs of PLLA-C(100) films before degradation (A) and after alkaline degradation for 25 h (B) and enzymatic degradation for 30 h (C).

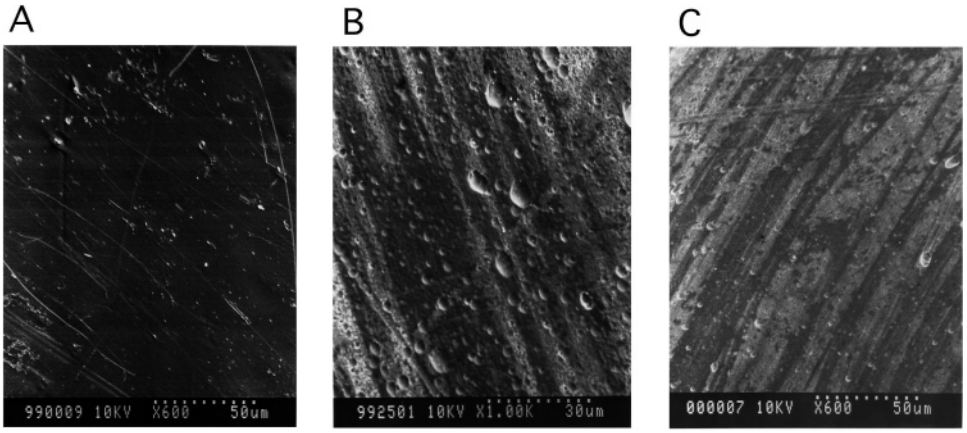

Figure 7. SEM photographs of PLLA-O films before degradation (A) and after alkaline degradation for 25 h (B) and enzymatic degradation for 30 h (C).

(2) The effects of biaxial orientation on the alkaline and autocatalytic degradation of the PLLA films were insignificant for the periods studied here. The X_c rather than the biaxial orientation seems to determine the alkaline and autocatalytic degradation rates of the PLLA films.

(3) The accumulation of crystalline residues formed as a result of selective cleavage and removal of the amorphous chains was observed for the de-oriented PLLA-C films, but not for the biaxially oriented PLLA-O film, when degraded in the presence of proteinase K. This means the facile release of formed crystalline residues from the biaxially oriented PLLA-O film during enzymatic degradation, due to the fact that the crystalline regions of PLLA-O film were oriented with their *c* axis parallel to the film surface.

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