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Poly(L-lactide): 7. Enzymatic hydrolysis of free and restricted amorphous regions in poly(L-lactide) films with different crystallinities and a fixed crystalline thickness

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Abstract

Poly(L-lactide) (PLLA) films having different initial crystallinities (x_c) (0–57%) and a fixed crystalline thickness were prepared by annealing the melt at a fixed temperature for different times. Their enzymatic hydrolysis was investigated in the presence of Proteinase K®. The rate of weight loss decreased rapidly and slowly with an increase in the initial x_c for x_c below and above 33%, respectively, where the free and the restricted amorphous regions, respectively, are the major amorphous components in the PLLA films. This is ascribed to the higher hydrolysis-resistance of the PLLA chains in the restricted amorphous region than that in the free amorphous region. Gel permeation chromatography (GPC) results revealed that in the restricted amorphous region the folding chains are much more hydrolysis-resistant than the tie chains and the chains with free ends. The increased x_c during the enzymatic hydrolysis is due to the preferential hydrolysis and removal of the amorphous regions, but not to the crystallization of the amorphous regions.

Keywords: Poly(L-lactide); Poly(L-lactic acid); Enzymatic hydrolysis

1. Introduction

Of the family of poly(L-lactide) aliphatic polyesters, (PLLA) has been intensively studied because of its biodegradability, compostability, non-toxicity, and high mechanical properties comparable with those of polyethylene and polystyrene [1–9]. Numerous investigations have been performed on the non-enzymatic hydrolysis of PLLA [1–9]. The environmental application of PLLA, however, requires fundamental information concerning its enzymatic hydrolysis. Despite the importance of this information, surprisingly little is available about its enzymatic hydrolysis [10–15].

Since Williams found that the hydrolysis behavior of PLLA is accelerated in the presence of Proteinase K [10], hydrolysis of PLLA has been studied using Proteinase K [11–14]. It was found that the enzymatic hydrolysis rate of PLLA becomes lower with increasing initial crystallinity (x_c) [11–13], D-lactate unit (half of lactide unit) composition, and the distribution of the two isomeric units [14]. These results suggest that the enzymatic hydrolysis is faster in the amorphous region than in the crystalline region. Recently, Iwata and Doi studied the enzymatic hydrolysis of PLLA single crystals using Proteinase K and concluded that their enzymatic hydrolysis proceeds at the disordered chain packing region of the crystal edges rather than at their chain folding surfaces [15].

In these previous studies the weight loss of PLLA specimens was mainly monitored and other information concerning the changes in molecular characteristics and ordering was not available in spite of its importance in elucidating the enzymatic hydrolysis mechanism. In addition, the hydrolizability was explained only in relation to the the gross crystallinity x_c without discussing the effects of the free and restricted amorphous regions on the enzymatic hydrolysis separately. The free amorphous region formed outside the spherulites should show a hydrolysis behavior different from the restricted amorphous region formed between the crystalline regions inside the spherulites because of the different molecular mobility.

The purpose of the present study is to investigate the enzymatic hydrolysis of the free and restricted amorphous regions of PLLA using Proteinase K. For this purpose, various PLLA films having a wide variety of x_c (0–57%) were prepared by annealing at a fixed temperature (T_a) for different times (t_a). The thickness (L_c) of the PLLA crystals could be made constant by fixing the T_a and melting

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Table 1
Characteristics of 50 μm-thick PLLA films before and after enzymatic hydrolysis

Code	Before hydrolysis				After hydrolysis				
	M_w ($\times 10^5$)	M_w/M_n	T_m (°C)	x_c (%)	Hydrolysis time (h)	M_w ($\times 10^5$)	M_w/M_n	T_m (°C)	x_c (%)
PLLA0	4.03	1.73	178	0	4	3.95	1.50	178	0
PLLA10	4.67	1.69	179	14	15	1.20	6.52	173	60
PLLA15	4.54	1.72	180	33	20	0.89	5.62	174	69
PLLA30	4.52	1.72	181	50	20	1.28	6.61	175	90
PLLA45	4.53	1.67	182	57	20	0.95	5.69	174	88

temperature (T_m)[16] to remove the L_c effect on the enzymatic hydrolysis of the two amorphous regions. The PLLA films remaining after enzymatic hydrolysis were analyzed by gravimetry, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and polarizing microscopy.

2. Experimental

2.1. Materials

The original PLLA films were prepared by casting a 1 g/dl solution of PLLA [Polysciences, weight-average molecular weight (M_w) = 3×10^5] in methylene chloride [17,18]. Each of the PLLA films was sealed in a glass tube under a reduced pressure, melted at 200°C for 3 min, and annealed at 140°C for different t_a. The film was then quenched at 0°C to stop further crystallization. The PLLA films annealed for 0, 10, 15, 30 and 45 min at 140°C are abbreviated as PLLA0, PLLA10, PLLA15, PLLA30, and PLLA45 films, respectively.

The characteristics of these annealed PLLA films are tabulated in Table 1. As can be seen, prolongation of annealing increased x_c from zero to 57% without a large variation of T_m and therefore L_c (see for example Ref. [16]). Our previous study showed that the minimum x_c was 39% for the PLLA film to consist of only restricted amorphous regions without a free amorphous region [17]. Therefore, PLLA30 and PLLA45 films contained the restricted amorphous region as the major amorphous part, while PLLA0 and PLLA10 films contained a significant ratio of the free amorphous region.

2.2. Hydrolysis

A PLLA film (10 mm × 10 mm × 50 or 25 μm) was placed in a vial filled with 5 ml of Tris–HCl buffer solution (pH 8.6) containing 1.0 mg of Proteinase K (Sigma, lyophilized powder, 80% protein) and 1.0 mg of sodium azide (Nacalai Tesque, the guaranteed grade) [12,18]. The solution pH remained in the range of 8.6–8.0 within the 20 h of the enzymatic hydrolysis, where the enzyme activity was reported to be practically constant [12]. The hydrolyzed PLLA film was washed thoroughly with distilled water at 4°C, followed by drying under a reduced pressure for at least two weeks [18]. The characteristics of the PLLA films after enzymatic hydrolysis are also presented in Table 1.

2.3. Measurements

The M_w and number-average molecular weight (M_n) of the PLLA films were evaluated in chloroform at 40°C by a Tosoh GPC system (refractive index monitor: RI-8020) with TSK Gel columns ($GMH_{XL} \times 2$) using polystyrene as a standard.

The temperatures (T_c and T_m) and enthalpies of crystallization and melting (ΔH_c and ΔH_m) of the PLLA films (sample weight of ca. 2 mg) were determined with a Shimadzu DT-50 differential scanning calorimeter at a heating rate of 10°C/min under a nitrogen gas flow at a rate of 50 ml/min. T_c, T_m, ΔH_c, and ΔH_m were calibrated using benzophenone, indium, and tin as standards. The x_c of the PLLA films was evaluated according to the following equation [17]:

$$x_c(\%) = 100(\Delta H_m + \Delta H_c)/93 \quad (1)$$

where 93 J/g is the ΔH_m of PLLA crystals having infinite

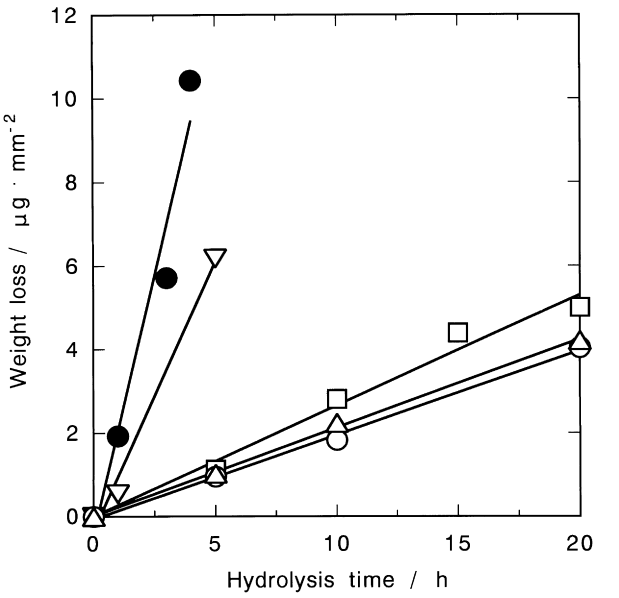

Fig. 1. Weight losses per unit surface area of PLLA0 (●), PLLA10 (▽), PLLA15 (□), PLLA30 (△), and PLLA45 (○) films as a function of enzymatic hydrolysis time.

crystal thickness reported by Fischer et al. [19]. By definition ΔH_m and ΔH_c are positive and negative, respectively. The morphology of the PLLA films was studied with a Zeiss polarizing microscope for samples of 25 μm thickness.

3. Results and discussion

3.1. Weight loss

The weight losses per unit surface area of the PLLA films with different x_c are plotted in Fig. 1 as a function of enzymatic hydrolysis time. Obviously, all the films show a linear weight loss with hydrolysis time without any induction period, which is comparable with the result reported for PLLA films hydrolyzed in a dilute alkaline solution [20]. The hydrolysis rate (R_{EH}) calculated from Fig. 1 is plotted in Fig. 2 against initial x_c of the PLLA films. Apparently, R_{EH} decreases monotonically with x_c, in good agreement with the results reported previously [11–13]. The reduced R_{EH} for the PLLA films having high x_c can be ascribed to the decreased fraction of the amorphous region in the surface. The R_{EH} of the PLLA0 film composed solely of the free amorphous region was 2.5 μg/mm^2 h, which is comparable with 2.4 μg/mm^2 h reported for a completely amorphous PLLA film by Li and McCarthy [13].

The slope of the tangent line of R_{EH} against x_c is much larger in the x_c range of 0–33% than in the x_c range of 33–57%, meaning that x_c has large and small effects on R_{EH} at low and high x_c, respectively. This finding suggests that the polymer chains in the restricted amorphous region are much more hydrolysis-resistant than those in the free amorphous region. The restricted amorphous region contains three types of polymer chains: (1) tie chains, (2) folding chains, and (3) chains with free ends. The high hydrolysis-resistance of the folding chains may cause the lowered enzymatic hydrolysis rate of the restricted amorphous region. On the other hand, the linear dependence of the alkaline hydrolysis rate of the PLLA films on their initial x_c for the similar x_c range (0–54%) [20] is probably due to the random or non-selective hydrolytic scission of the chains in these amorphous regions.

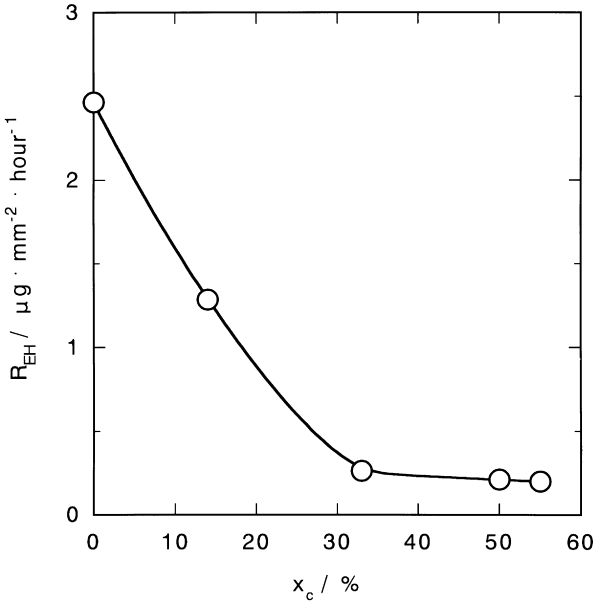

Fig. 2. R_{EH} of PLLA films as a function of initial x_c.

3.2. Molecular weight change

No significant change was recognized in the GPC curves of the PLLA0 films before and after enzymatic hydrolysis for 4 h, whereas the PLLA45 film having the restricted amorphous region showed a complicated change as illustrated in Fig. 3. The main peak at a molecular weight of 2×10^5 became smaller with the progress of enzymatic hydrolysis without changing its position. The multiple peaks shown at molecular weights of ca. 7.5×10^3, 1.5×10^4 and 2.5×10^4 are abbreviated as Peaks I, II, and III, respectively. The formation of these peaks is ascribed to the preferential hydrolytic scission and removal of the tie chains and the chains with free ends in the restricted amorphous regions, leaving the PLLA crystalline residues. The areas of these three peaks became higher with hydrolysis time, without a

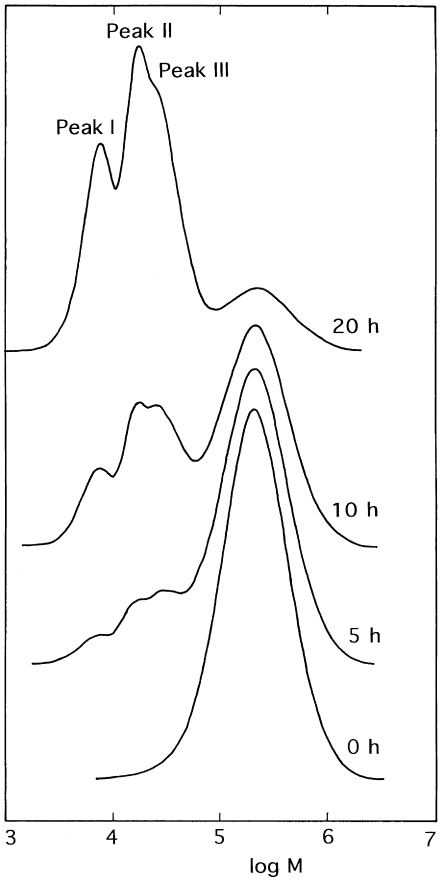

Fig. 3. GPC curves of PLLA45 films before and after enzymatic hydrolysis for different times.

change in the molecular weight. This finding reveals that the folding chains in the restricted amorphous region are much more hydrolysis-resistant than the tie chains and the chains with free ends. In contrast, the lowest-molecular-weight specific peak alone remained after long-term non-enzymatic hydrolysis of crystallized PLLA specimens in vitro [20–23] and in vivo [24], due to the random scission and removal of the chains in the restricted amorphous region.

3.3. Changes in the crystalline and the amorphous regions

The x_c and T_m of the PLLA films are plotted in Fig. 4(a) and (b), respectively, as a function of enzymatic hydrolysis time. x_c of the initially amorphous PLLA0 film remains practically zero throughout the hydrolysis time of 4 h, revealing that no significant crystallization occurred in the free amorphous region. In contrast, x_c increases monotonically upon enzymatic hydrolysis of all the crystallized PLLA films. This increased x_c is probably due to the predominant hydrolytic removal of the chains in both the free and the restricted amorphous regions, but not to the PLLA crystallization of the amorphous region. A similar increase in crystallininity is observed in the long-term non-enzymatic hydrolysis of the amorphous [21,22,25] and the crystallized [20,22–27] PLLA films, which is attributed to the selective hydrolytic removal and the crystallization of the PLLA chains in both the free and the restricted amorphous regions.

As seen in Fig. 4(b), the initial T_m of all the PLLA films is constant below 10 h hydrolysis time. A new lower T_m appears for all the PLLA films except for the amorphous PLLA0 film when the hydrolysis exceeds 10 h. The new T_m is also retained at a constant value. This is in marked contrast with the result of the PLLA films hydrolyzed non-enzymatically in phosphate-buffered solution, where an initial increase in T_m occurred, followed by a gradual decrease for longer hydrolysis times [22,23]. The lower T_m is ascribed to the PLLA crystalline residues formed by the enzymatic hydrolysis.

The polarizing photomicrographs of PLLA30 films before and after enzymatic hydrolysis for 5 h are given in Fig. 5. The area covered with the spherulites is rather smaller than that expected from the x_c value of PLLA30 film, because the photo was taken for the selected area having the lowest spherulite density. The decreased photographic contrast in the spherulites after enzymatic hydrolysis means the disorientation of the PLLA lamellae by hydrolysis and removal of the tie chains in the restricted

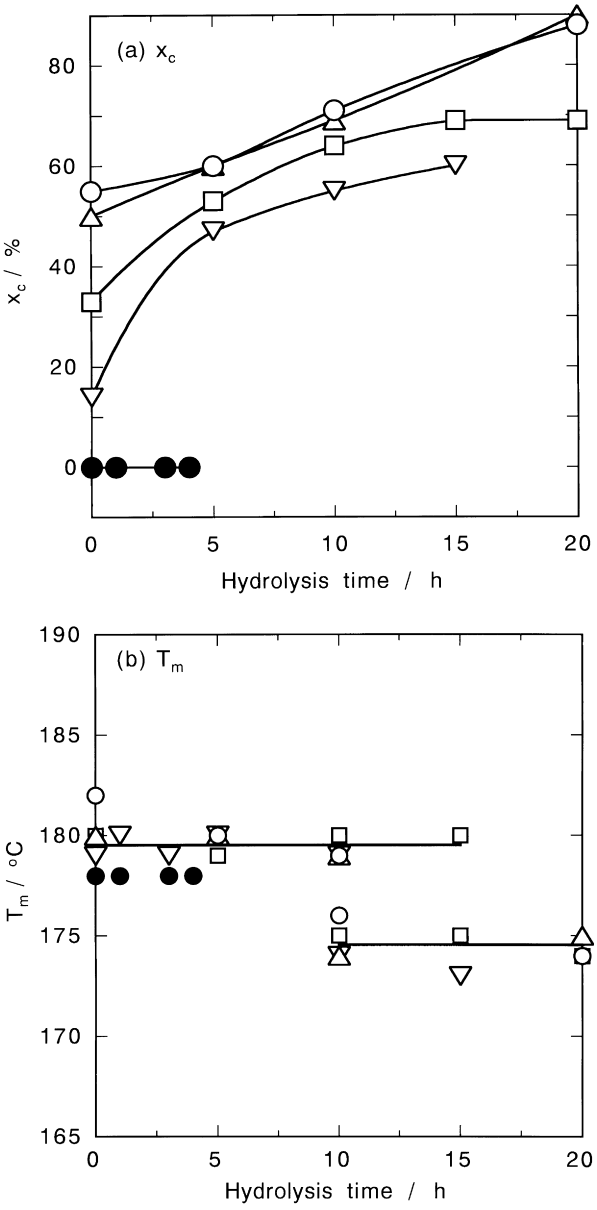

Fig. 4. x_c (a) and T_m (b) of PLLA0 (●), PLLA10 (▽), PLLA15 (□), PLLA30 (△), and PLLA45 (○) films as a function of enzymatic hydrolysis time.

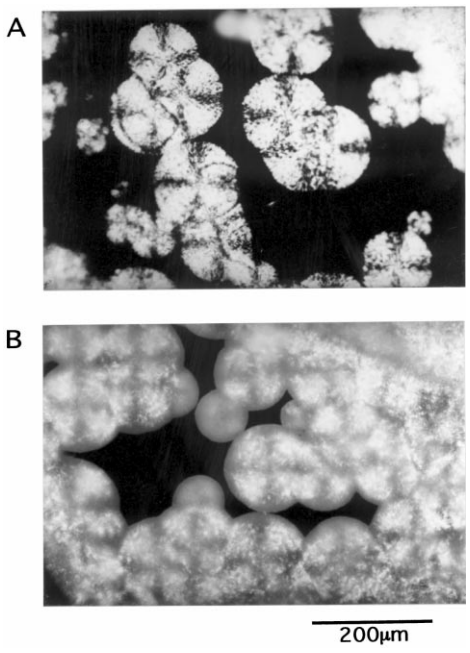

Fig. 5. Polarizing photomicrographs of PLLA30 films before (A) and after enzymatic hydrolysis for 5 h (B).

amorphous region. An analogous result was reported for the PLLA films hydrolyzed non-enzymatically in a dilute alkaline solution [20].

4. Conclusions

From the above results, we conclude that the hydrolysis-resistance of PLLA chains in the restricted amorphous region is higher than that in the free amorphous region, and that among the chains in the restricted amorphous region the folding chains are much more hydrolysis-resistant than the tie chains and the chains with free ends, resulting in the reduced average enzymatic hydrolysis rate in the restricted amorphous region. Moreover, the increased x_c during enzymatic hydrolysis is ascribed to preferential hydrolysis and removal of the chains in both the free and the restricted amorphous regions compared with those in the crystalline region, but not to the crystallization in these amorphous regions during the enzymatic hydrolysis.

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