Poly(L-lactide). X. Enhanced Surface Hydrophilicity and Chain-Scission Mechanisms of Poly(L-lactide) Film in Enzymatic, Alkaline, and Phosphate-Buffered Solutions

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Received 19 March 2002; accepted 29 May 2002

ABSTRACT: Attempts were carried out to enhance the surface hydrophilicity of poly(L-lactide), that is, poly(L-lactic acid) (PLLA) film, utilizing enzymatic, alkaline, and autocatalytic hydrolyses in a proteinase K/Tris–HCL buffered solution system (37°C), in a 0.01*N* NaOH solution (37°C), and in a phosphate-buffered solution (100°C), respectively. Moreover, its chain-scission mechanisms in these different media were studied. The advancing contact-angle (θ_a) value of the amorphous-made PLLA film decreased monotonically with the hydrolysis time from 100° to 75° and 80° without a significant molecular weight decrease, when enzymatic and alkaline hydrolyses were continued for 60 min and 8 h, respectively. In contrast, a negligible change in the θ_a value was observed for the PLLA films even after the autocatalytic hydrolysis was continured for 16 h, when their bulk M_n decreased from 1.2×10^5 to 2.2×10^4 g mol^{-1} or the number of hydrophilic terminal groups per unit weight increased from 1.7×10^{-5} to 9.1×10^{-5} mol g^{-1}. These findings, together with the result of gravimetry, revealed that the enzymatic and alkaline hydrolyses are powerful enough to enhance the practical surface hydrophilicity of the PLLA films because of their surface-erosion mechanisms and that its practical surface hydrophilicity is controllable by varying the hydrolysis time. Moreover, autocatalytic hydrolysis is inappropriate to enhance the surface hydrophilicity, because of its bulk-erosion mechanism. Alkaline hydrolysis is the best to enhance the hydrophilicity of the PLLA films without hydrolysis of the film cores, while the enzymatic hydrolysis is appropriate and inappropriate to enhance the surface hydrophilicity of bulky and thin PLLA materials, respectively, because a significant weight loss occurs before saturation of θ_a value. The changes in the weight loss and θ_a values during hydrolysis showed that exo chain scission as well as endo chain scission occurs in the presence of proteinase K, while in the alkaline and phosphate-buffered solutions, hydrolysis proceeds via endo chain scission. J Appl Polym Sci 87: 1628–1633, 2003

Key words: biodegradable; biomaterials; degradation; hydrophilic polymers; polyesters

INTRODUCTION

Poly(L-lactide), that is, poly(L-lactic acid) (PLLA), is an aliphatic polyester which is producible from renewable resources, very low or nontoxic in natural environments, biodegradable, and compostable. Moreover, PLLA has a high mechanical performance comparable to that of commercial polymers such as polyethylene, polypropylene, and polystyrene.[1–10] The hydrolysis of PLLA materials is reported to be accelerated by the presence of external catalysts such as enzymes and alkalis.[7–10] In the presence of the external catalysts such as enzymes and alkalis, the hydrolysis of PLLA materials proceeds mainly via a surface-erosion mechanism. In contrast, without external catalysts, as in a phosphate-buffered solution, the hydrolysis of PLLA materials takes place via a bulk-erosion mechanism.

Proteinase K is a well-known enzyme to catalyze the hydrolysis of PLLA materials[11] and the effects of the molecular characteristics and highly ordered structures on proteinase K-catalyzed hydrolysis of polylactides, that is, poly(lactic acid)s (PLAs), have been intensively studied.[12–26] In alkaline media, the hydrolysis of PLLA microspheres is accelerated, as Makino et al. reported,[27] and the effects of highly ordered structures such as crystallinity,[28,29] crystalline thickness,[29] and spherulite size[29] on the hydrolysis of PLLA materials have been studied. The hydrolysis of PLLA materials without external catalysts as in a phosphate-buffered solution is believed to proceed autocatalytically by the terminal carboxyl groups.[7–10] The effects of numerous structural parameters of molecular characteristics and highly ordered structures on the autocatalytic hydrolysis of PLLA materials have been studied.[7–10] However, a detailed chain-scission mechanism in PLLA films in different hydrolytic media has not been reported so far.

In addition, despite the above-mentioned intensive studies, limited information is available for the surface

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Contract grant sponsor: Ministry of Education, Culture, Sports, Science and Technology (Japan); contract grant number: 11217209.

Journal of Applied Polymer Science, Vol. 87, 1628–1633 (2003)

TABLE I
Characteristics of Melt-quenched PLLA Film

M_n (g/mol)	M_w/M_n	T_g (°C)	T_c (°C)	T_m (°C)	x_c (%)	θ_a (degrees)	θ_r (degrees)
1.2×10^5	2.1	54	123	172	0	101	59

hydrophilicity change during the hydrolysis in the different media and the utilizability of the hydrolysis to enhance the surface hydrophilicity. Controlling the surface hydrophilicity of biodegradable and biomedical materials is crucial in controling their biodegradability[30] and biocompatibility,[31] respectively.

The purposes of this study were to attempt to enhance the surface hydrophilicity of PLLA film utilizing hydrolysis in different hydrolysis media and to investigate their chain-scission mechanisms by the measurements of surface hydrophilicity. For these purposes, the surface hydrophilicity and hydrolysis of the amorphous-made PLLA films after hydrolysis in a proteinase K/Tris–HCl-buffered solution system, in a NaOH solution (37°C), and in a phosphate-buffered solution (100°C) were monitored using dynamic contact-angle measurements, gravimetry, and gel permeation chromatography.

EXPERIMENTAL

Materials

PLLA (LACTY^{TM}5000) was kindly supplied by the Shimadzu Co. (Kyoto, Japan) and purified by precipitation using methylene chloride and methanol as the solvent and nonsolvent, respectively. As-cast PLLA film prepared using methylene chloride as a solvent were made amorphous as follows[22]: Each of the films was placed between two Teflon sheets and then sealed in a glass tube under reduced pressure. The sealed films were melted at 200°C for 3 min and then quenched at 0°C. The films for autocatalytic hydrolysis in a phosphate-buffered solution at 97°C were melted at 200°C for 3 min and crystallized at 100°C for 600 min to avoid changes in highly ordered structures and a large thermal deformation during high-temperature hydrolysis.

Hydrolysis

The enzymatic hydrolysis of the amorphous-made films ($10 \times 25 \times 200$ μm) was performed according to the procedure reported by Reeve et al.,[12] namely, each of the films was placed in a vial filled with 10 mL of a Tris–HCl buffered solution (pH 8.6 ± 0.1) containing 2.0 mg of proteinase K (Sigma, lyophilized powder, 80 % protein) and 2.0 mg of sodium azide (guaranteed grade, Nacalai Tesque Inc., Kyoto, Japan). The enzymatic hydrolysis of the films was performed at 37°C in a rotary shaker for up to 120 min. The solution pH remained in the range between 8.6 and 8.0 in 120 min, where the enzyme activity was reported to be practically constant.[12]

The alkaline and autocatalytic hydrolyses of each of the amorphous-made and crystallized films ($10 \times 25 \times 200$ μm) were carried out in a vial filled with 10 mL of a 0.01*N* NaOH solution (pH 11.9 ± 0.1, Nacalai Tesque Inc., Kyoto, Japan) and of a phosphate-buffered solution (pH 7.4 ± 0.1) at 37 and 97°C for up to 24 and 16 h, respectively.

The hydrolyzed films were washed thoroughly with distilled water at 4°C to stop further hydrolysis, followed by drying under reduced pressure for at least 2 weeks. The distilled water used for the preparation of the buffered solutions and washing of the hydrolyzed films was of HPLC grade (Nacalai Tesque Inc.). After complete drying, the films hydrolyzed in the phosphate-buffered solution were amorphous-made by the procedure mentioned above to remove the effects of the crystalline region on the hydrophilicity.

Measurements

The weight- and number-average molecular weights (M_w and M_n, respectively) and the molecular weight distribution of the amorphous-made film 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 crystallization and melting temperatures (T_c and T_m, respectively) and the enthalpies of crystallization and melting (ΔH_c and ΔH_m, respectively) of the melt-quenched film were determined by a Shimadzu DT-50 differential scanning calorimeter. The film (sample weight of ca. 2 mg) was heated at a 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 T_m of the melt-quenched film is shown in Table I. The x_c of the film was evaluated according to the following equation[22,32]:

$$x_c\ (\%) = 100 \times (\Delta H_m + \Delta H_c)/135 \qquad (1)$$

where 135 J/g is the ΔH_m of the PLLA crystal having an infinite size reported by Miyata and Masuko.[33] By definition, ΔH_m and ΔH_c are positive and negative, respectively. The DSC measurements exhibited that

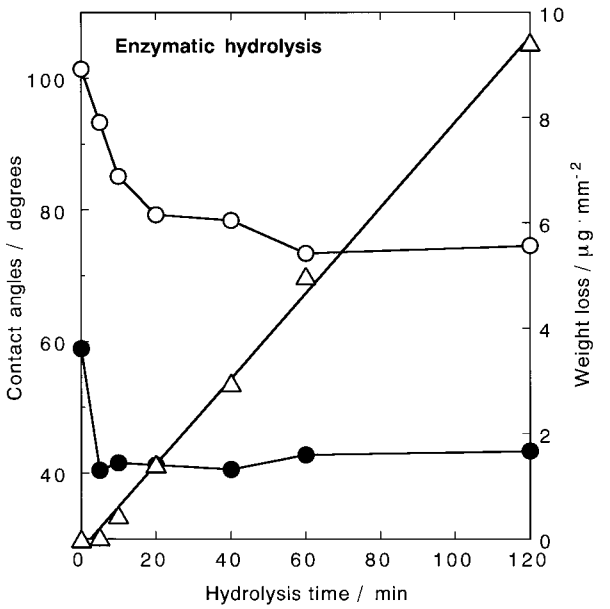

Figure 1 Advancing and receding contact angles [(○) θ_a and (●) θ_r, respectively] and (△) weight loss per unit surface area of PLLA film hydrolyzed enzymatically in the presence of proteinase K as a function of hydrolysis time.

the PLLA film was completely amorphous after melt-quenching. The characteristics of the amorphous-made film before hydrolysis are given in Table I.

The advancing and receding contact angles (θ_a and θ_r, respectively) of the films before and after hydrolysis in the different media were measured by the Wilhelmy plate method[34] using a DCA-100 (A&D Co. Ltd., Tokyo, Japan). The measurements were performed in distilled water (HPLC grade, Nacalai Tesque Inc.) at 25°C and a film speed of 20 mm/min. Contact angles are indexes for the net density of hydrophilic terminal carboxyl and hydroxyl groups of the film surface. However, θ_r may be an inappropriate index because of the absorption of water during the first advancing measurement. Accordingly, θ_a is regarded as the main index for the hydrophilicity, but θ_r is also shown in the following figures for reference.

RESULTS AND DISCUSSION

Enzymatic hydrolysis

The changes in contact angles and weight loss per unit surface area of the PLLA film during enzymatic hydrolysis are shown in Figure 1. Obviously, the enzymatically hydrolyzed films showed a linear weight loss with the hydrolysis time without any induction periods. The θ_a value of the enzymatically hydrolyzed films decreased monotonically from 101° with the hydrolysis time and reached a plateau around 75° at 60 min, whereas their θ_r decreased dramatically from 60 to 40 degrees in 5 min and remained unchanged for the hydrolysis time exceeding 5 min. The θ_a value for the PLLA film before enzymatic hydrolysis (101°) is higher than the 95° reported for an amorphous-made PLLA film.[35] It is probable that a trace amount of a hydrophilic solvent 1,1,1,3,3,3-hexafluoro-2-propanol used in the previous study for casting and remaining after drying, increased the surface hydrophilicity of the PLLA film. The θ_a value for the PLLA film after the enzymatic hydrolysis for 120 min (75°) is slightly smaller than is the 61° for a hydrophilic poly(vinyl alcohol) (PVA) film, but is very close to 81° for a blend film from PLLA and PVA (50/50).[35] Tsuchiya et al. showed that the contact angle of a PLLA film can be decreased by 50° by the grafting of hydrophilic acrylamide chains.[36] The result obtained here indicates that the practical surface hydrophilicity of the PLLA films can be enhanced the enzymatic hydrolysis and that the hydrophilicity of the films is controllable by varying the enzymatic hydrolysis time.

The θ_a values of all the PLLA films after the enzymatic hydrolysis exceeding 5 min (73–93 degrees) were smaller than was the 105° for the PLLA film having an M_n as low as 2.2×10^4 g mol^{-1}, which is shown in Figure 3. The bulk molecular weight of the PLLA film remained unchanged after 120 min of enzymatic hydrolysis, in agreement with the result reported earlier.[22] These findings mean that the PLLA films having a practical surface hydrophilicity much higher than that of the PLLA film having a bulk M_n as low as 2.2×10^4 g·mol^{-1}, below which fragmentation of the films occurred, can be obtained by enzymatic hydrolysis without a significant change in the bulk molecular weight. The PLLA chains on the film surface after the enzymatic hydrolysis exceeding 5 min must have an M_n much lower than that of the PLLA film having an M_n of 2.2×10^4 g mol^{-1}. However, a percentage weight loss of film 200 μm thick after 120 min of enzymatic hydrolysis was 10.0 wt %, suggesting that enzymatic hydrolysis is appropriate and inappropriate to enhance the hydrophilicity of bulky and thin materials, respectively. The rapid weight loss and insignificant M_n decrease reflect a surface-erosion mechanism in the presence of proteinase K.

As seen in Figure 1, the weight loss of the PLLA film started to increase before saturation of the θ_a value. If only endo chain scission occurred, weight loss would have started to increase after a significant induction period until the saturation of the θ_a value. The endo chain scission of PLLA was found to occur in the presence of proteinase K in our previous study.[26] Therefore, the measurements of the surface hydrophilicity confirm that exo chain-scission as well as the endo one occurs during the hydrolysis of the PLLA films in the presence of proteinase K as suggested earlier.[26]

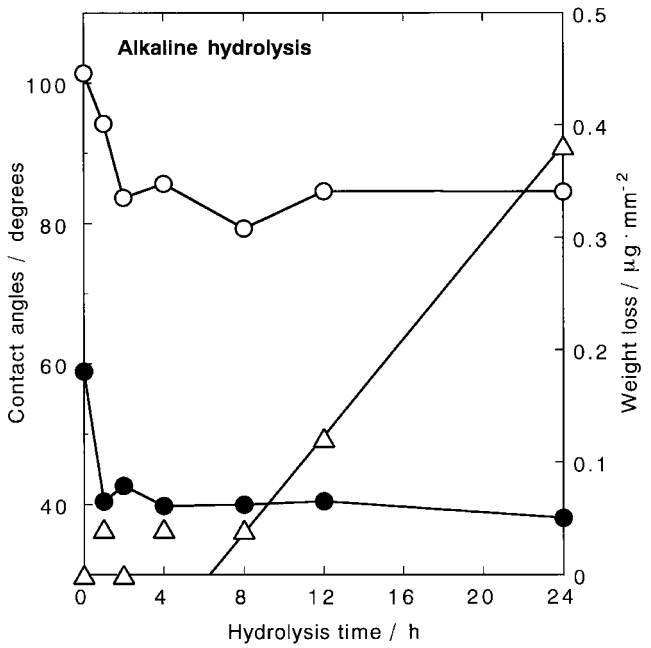

Figure 2 Advancing and receding contact angles [(○) θ_a and (●) θ_r, respectively] and (△) weight loss per unit surface area of PLLA film hydrolyzed catalytically in 4*N* NaOH solution as function of hydrolysis time.

Alkaline hydrolysis

The changes in contact angles and weight loss per unit surface area of the PLLA film during alkaline hydrolysis for different times are presented in Figure 2. The weight loss started to increase after a significant induction period of 8 h. In contrast, the θ_a value of the film decreased monotonously from 101° with the hydrolysis time without any induction periods and then reached a plateau around 80° at 8 h. This indicates that the practical surface hydrophilicity of the PLLA films can be increased by alkaline hydrolysis and that the practical surface hydrophilicity of the films is controllable by varying the alkaline hydrolysis time. The final θ_a value (85°) at 24 h is comparable with 88° of a PLLA film hydrolyzed in a 3.75*N* NaOH solution for a fixed time of 20 min.[37] On the other hand, the θ_r values of the PLLA film decreased rapidly from 60 to 40° in 1 h and remained unchanged for the hydrolysis period exceeding 1 h. A negligible weight loss (0.04 wt %) and a molecular weight decrease were observed for the PLLA film after the alkaline hydrolysis for 24 h. These findings mean that the alkaline hydrolysis is highly appropriate to enhance the surface hydrophilicity of thin materials as well as bulky ones, without decreasing the bulk molecular weight or the hydrolysis of the material core. The significant decrease in the contact angles and the insignificant molecular weight change reflect a surface-erosion mechanism in the alkaline solution. The rapid saturation of the θ_r value compared with that of θ_a value during the alkaline hydrolysis agrees with that during the enzymatic hydrolysis. This may be ascribed to the water absorption during the first advancing measurement.

In contrast to the result for the enzymatically hydrolyzed PLLA films, the weight loss in the alkaline solution started to increase after the saturation of the θ_a value. This strongly suggests that endo chain scission occurs during the alkaline hydrolysis, which is comparable with that reported for alkali-catalyzed hydrolysis of poly(DL-lactide), that is, poly(DL-lactic acid), in a *p*-dioxane solution.[38] If exo chain-scission occurred, a significant weight loss would have observed before saturation of the θ_a value.

Autocatalytic hydrolysis

The changes in the contact angles and the M_n of the PLLA film during autocatalytic hydrolysis in the phosphate-buffered solution are given in Figure 3. The M_n of the PLLA film decreased monotonically with the hydrolysis time from 1.2×10^5 to 2.2×10^4 g mol^{-1} without a change in the molecular weight distribution (M_w/M_n = 2.0–2.1). In other words, the molecular weight distribution of the PLLA film shifted to a lower molecular weight as a whole with the autocatalytic hydrolysis time (data not shown).[39] This result confirms that endo chain scission occurs in the phosphate-buffered solution. The fragmentation of the film, which made the contact-angle measurements impossible, occurred at 20 h. The weight loss of the film was very small (3.2 wt %) even after 16 h of hydrolysis,

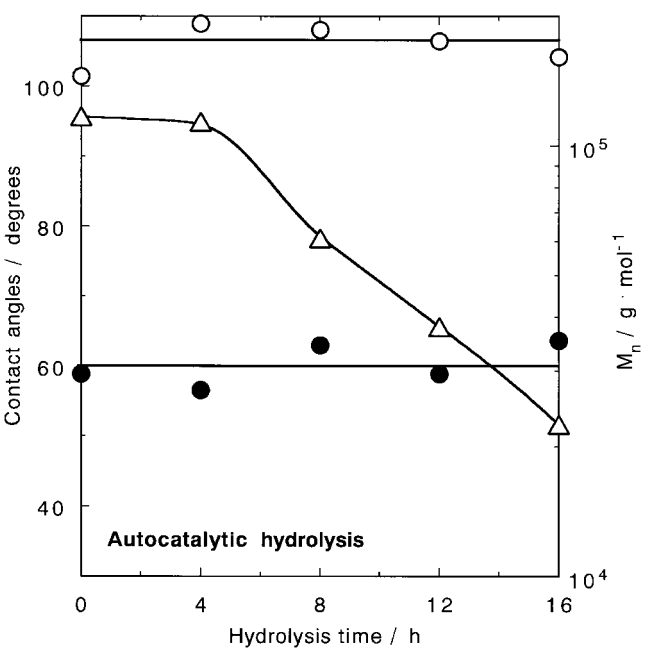

Figure 3 Advancing and receding contact angles [(○) θ_a and (●) θ_r, respectively] and M_n (△) of PLLA film hydrolyzed autocatalytically in a phosphate-buffered solution as a function of hydrolysis time.

TABLE II
Hydrolysis Mechanisms of PLLA Film in Different Media

Hydrolysis media	Hydrolysis mechanisms	
	Chain scission	Material erosion
In the presence of proteinase K (1 mg/5 mL, pH 8.6 ± 0.1)	Endo and exo	Surface
Alkaline solution (pH 11.9 ± 0.1)	Endo	Surface
Phosphate-buffered solution (pH 7.4 ± 0.1)	Endo	Bulk

when the large M_n decrease occurred. These findings reflect that the autocatalytic hydrolysis of the PLLA film in a phosphate-buffered solution proceeds via a bulk-erosion mechanism.

The θ_a and θ_r values of the film were practically constant even when the M_n decreased from 1.2×10^5 to 2.2×10^4 g·mol^{-1}. The hydrolytic chain scission of ester groups increased the number of hydrophilic terminal carboxyl and hydroxyl groups per unit weight from 1.7×10^{-5} [2/(1.2×10^5)] to 9.1×10^{-5} [2/(2.2×10^4)] mol g^{-1} in 16 h. Here, two of numerators in the parentheses is the number of terminal groups (one carboxyl and one hydroxyl group) which one PLLA molecule has and the values of denominators in the parentheses are M_n values before and after the autocatalytic hydrolysis for 16 h. Thereby, it became evident that the small increase in the number of hydrophilic terminal groups per unit weight from 1.7×10^{-5} to 9.1×10^{-5} mol g^{-1} has a negligible effect on the surface hydrophilicity of the PLLA film. The hydrolysis mechanisms of PLLA at the molecular and material levels are summarized in Table II. The effects of the surface hydrophilicity on the biodegradation and biocompatibility will be published in the near future.

CONCLUSIONS

From above-mentioned results, the following conclusions can be derived for the enhancement of the surface hydrophilicity and the chain-scission mechanisms of the PLLA film in different media:

1. The enzymatic and alkaline hydrolyses are powerful enough to enhance the practical surface hydrophilicity of the PLLA films because of their surface-erosion mechanisms. The hydrophilicity of the PLLA films is controllable in the θ_a range of 75–100 degrees using enzymatic and alkaline hydrolyses by varying the hydrolysis time. The alkaline surface hydrolysis is best to enhance the hydrophilicity of PLLA films without hydrolysis of the film cores, while the enzymatic surface hydrolysis is appropriate and inappropriate to enhance the hydrophilicity of bulky and thin materials, respectively, because a significant weight loss occurred before saturation of the θ_a value.
2. A negligible hydrophilicity change of the PLLA film occurred even when the M_n value was decreased from 1.2×10^5 to 2.2×10^4 g mol^{-1} or the number of hydrophilic terminal groups per unit weight increased from 1.7×10^{-5} to 9.1×10^{-5} mol g^{-1}. This means that the autocatalytic bulk hydrolysis is inappropriate to enhance the surface hydrophilicity of the PLLA films.
3. Both endo and exo chain scission occurs during the enzymatic hydrolysis in the presence of proteinase K, whereas the exo chain scission occurs during the alkaline and autocatalytic hydrolyses.

This research was partly supported by a Grant-in-Aid for Scientific Research on Priority Area, "Sustainable Biodegradable Plastics" (No. 11217209), from the Ministry of Education, Culture, Sports, Science and Technology (Japan).

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