Influence of Crystallinity and Stereochemistry on the Enzymatic Degradation of Poly(lactide)s

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Introduction

Polylactide or PLA has been investigated worldwide as a degradable biomaterial due to its excellent biocompatibility and variable degradability.[1−3] Attention is also paid to PLA polymers with respect to the problems of solid waste accumulation.[4,5] Because of the chirality of lactyl unit, lactide exists in three diastereoisomeric forms: L-lactide, D-lactide, and *meso*-lactide. Another widely used lactide, namely DL-lactide, is composed of equimolar L- and D-lactides. PLA homo- and stereocopolymers with dramatically different properties can be obtained by ring-opening polymerization of different lactide monomer feeds. The abbreviation PLA_x was introduced to designate these polymers with *x* reflecting the percentage of L-lactyl units in the feed.

The hydrolytic degradation mechanism of PLA polymers has been well established. It has been previously shown that degradation of PLA massive devices is heterogeneous: faster inside than at the surface.[6,7] This was assigned to an internal autocatalytic effect of carboxyl end groups. Degradation of PLA polymers in soil under natural conditions has also been investigated.[8] It was concluded that these polymers are bioassimilable; i.e. degradation byproducts resulting from the hydrolytic degradation can be totally assimilated by microorganisms such as fungi or bacteria. Therefore, PLA polymers can be considered as environmentally friendly materials.

The enzymatic degradation of PLA polymers has been investigated by a number of authors. A specific enzyme, proteinase K, has been shown to have significant effects on PLA degradation.[9−11] Reeve et al. observed that proteinase K preferentially degraded L-lactyl units as opposed to D-lactyl ones, PLA_0 being undegradable.[12] The maximum degradation rate was found for PLA_{92}. The authors suggested a critical disruption of the crystalline order resulted from the introduction of 8% D-lactyl units.

Further investigations were carried out by MacDonald et al. and Cai et al.[13,14] MacDonald et al. observed that amorphous films of PLA_x derived from L-/D-lactides with *x* ranging from 80 to 95 exhibited almost identical weight loss rates, in contrast to Reeve et al.'s results showing a strong increase of weight loss rate from PLA_{50} to PLA_{92}. On the other hand, PLA_x derived from L-/*meso*-lactides showed lower weight loss rates than those of the L-/D-lactides series. This finding was related to an inhibiting effect of L−D−L triads present in PLA_x from L-/*meso*-lactides.[13] Recently, Cai et al. found that the degradation rate of PLA_{96} decreased with increase in crystallinity. A threshold was observed when the heat of fusion was less than 20 J/g.[14]

It seems that the literature presents a rather complete pattern as far as the effects of crystallinity and stereochemistry on the enzymatic degradation of PLA_x stereocopolymers are concerned. Nevertheless, controversial and confusing data still exist. In Reeve et al.'s work, for example, the unannealed PLA_{100} showed much slower weight loss rate than PLA_{92} despite the lower crystallinity of the former (27% for PLA_{100} vs 32% for PLA_{92}). The initial surface degradation rate was almost the same for PLA_{75}, PLA_{92}, and unannealed PLA_{100}, but the weight loss rates were very different.[12] In MacDonald et al.'s work, the difference between the weight loss rates of the two series of PLA_x was explained by the presence of L−D−L triads acting as inhibitor.[13] However, other parameters could also have been the source of discrepancies. For example, the concentration of initiator used to polymerize L-/D-lactides was 10 times higher than that for the L-/*meso*-lactides series. Last, but not least, it is difficult to compare the results obtained by Reeve et al., MacDonald et al., and Cai et al. due to the involvement of many uncontrolled factors such as origin of polymers, film preparation method, film thickness, rotation rate, retention of enzymatic activity, etc.[12−14]

In this work, we have investigated the enzymatic degradation characteristics of two representative PLA polymers, namely PLA_{100} and PLA_{50}. The results obtained from weighing, DSC, and SEC are reported herein and discussed in comparison with literature data.

Experimental Section

Materials. Poly(L-lactide) and poly(DL-lactide), namely PLA_{100} and PLA_{50}, were obtained from Bohringer Inhelgeim Co. and Birmingham Polymers Co., respectively. The two polymers were purified by the dissolution/precipitation method using chloroform as solvent and ethanol as nonsolvent. Films of 0.3 mm thickness were prepared by compression molding at 190 and 120 °C, respectively, followed by rapid cooling. PLA_{100} films were annealed at 105 °C for 0, 1, 2, 3, and 60 min. Specimens with dimensions 10 × 10 × 0.3 mm^3 which weighed about 40 mg were then cut from the films.

Tris/HCl buffer solution with pH = 9.0, proteinase K in the form of lyophilized powder, and sodium azide were all supplied by Sigma.

Enzymatic Degradation. For degradation studies, each specimen was placed into a vial filled with 5 mL of Tris/HCl buffer solution containing 1.0 mg of proteinase K and 1.0 mg of sodium azide. The vials were allowed to rotate at 150 rpm in a rotary shaker thermostated at 37 °C. The solution was changed after 30 and 54 h to restore the original level of enzymatic activity. For a given experiment, three replicate samples were withdrawn and washed with distilled water. After wiping, the samples were weighed, vacuum-dried at room temperature for 1 week, and weighed again. Experimental weight loss and water uptake values represent averages of measurements from three replicate samples.

Measurements. Differential scanning calorimetry (DSC) thermograms were registered with a DuPont DSC 912 calorimeter, the heating rate being 10 °C/min. A 10 mg sample of material was used for each analysis. Size-exclusion chromatography (SEC) measurements were performed on a Waters apparatus equipped with UV/RI detectors. $CHCl_3$ was used

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Table 1. Crystallinity Data of PLA_{50} and PLA_{100} Films

polymer	PLA_{50}	PLA_{100}-0	PLA_{100}-1	PLA_{100}-2	PLA_{100}-3	PLA_{100}-60
crystallinity[a] (%)	0	6.4	13.3	25.7	32.1	49.2
degradation rate (μg/mm^2 h)	1.9	2.2	2.1	2.1	1.6	0.3

[a] Deduced from DSC thermograms.

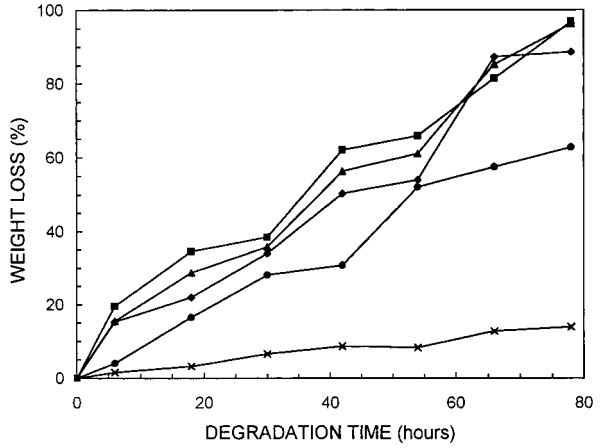

Figure 1. Weight loss changes of PLA_{100}-0 (■), PLA_{100}-1 (◆), PLA_{100}-2 (▲), PLA_{100}-3 (●), and PLA_{100}-60 (×) films during the enzymatic degradation at 37 °C.

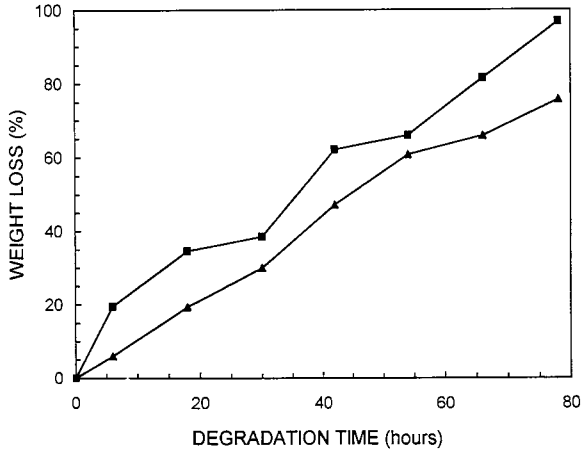

Figure 2. Weight loss changes of PLA_{100}-0 (■) and PLA_{50} (▲) films during the enzymatic degradation at 37 °C.

as mobile phase at a flow rate of 1.0 mL/min. Calibration was accomplished with polystyrene standards (Polysciences, USA).

Results

Both PLA_{100} and PLA_{50} were high molecular weight polymers. PLA_{100} initially exhibited weight-average molecular weight $\bar{M}_w$ = 164 000 with a rather narrow polydispersity ($\bar{M}_w/\bar{M}_n$ = 1.9). PLA_{50} showed initially $\bar{M}_w$ = 114 000 with $\bar{M}_w/\bar{M}_n$ = 1.6.

PLA_{50} is an intrinsically amorphous polymer with T_g = 50 °C as deduced from DSC. In contrast, PLA_{100} is intrinsically semicrystalline. It exhibited T_g = 55 °C and T_m = 175 °C. The different PLA_{100} samples are denoted PLA_{100}-*Y*, with *Y* representing the annealing time (in minutes). The degree of crystallinity (χ_c) was obtained by DSC using the following equation:

$$\chi_c\,\% = (\Delta H_m - \Delta H_c)/93 \qquad (1)$$

where ΔH_m is the melting enthalpy, ΔH_c the crystallization enthalpy, and 93 (J/g) the melting enthalpy of totally crystallized PLA_{100}.[15] The crystallinity data as deduced from DSC are presented in Table 1.

Weight loss data of the various PLA_{100} samples are shown in Figure 1. It appeared that the percentage of weight loss depended on the crystallinity. For the highly crystalline PLA_{100}-60, weight loss increased very slowly to attain 14% after 78 h. PLA_{100}-3 of intermediate crystallinity exhibited a faster weight loss which reached 63% at the end of the degradation period. Finally for PLA_{100}-0, PLA_{100}-1, and PLA_{100}-2 of lower crystallinity, weight loss increased very fast to attain nearly 96% after 78 h. Little distinction could be made in the group of low-crystallinity PLA_{100} samples.

The effect of stereochemistry on the enzymatic degradation of PLA was evidenced by comparing the weight loss profiles of the two unannealed PLA samples, i.e. PLA_{100}-0 and PLA_{50}, as shown in Figure 2. PLA_{100}-0 degraded more rapidly than PLA_{50} despite the presence of traces of crystallinity. This finding confirmed that the enzyme preferentially degrades L-lactyl, as opposed to D-lactyl units.[12] At the end of the degradation period, PLA_{50} had lost about 76% of its initial weight, i.e., 20% less than PLA_{100}. Nevertheless, this difference appeared to be less than expected since with the presence of 50% of D-lactyl units, PLA_{50} should have degraded at half the rate of PLA_{100}-0. The degradation rate of the polymers was obtained from the slope of the linear regression of weight loss curves (Figures 1 and 2), as shown in Table 1. The degradation rate of PLA_{50} was only slightly lower than that of PLA_{100}-0.

The weight loss difference between PLA_{100}-0 and PLA_{50} seemed to occur during the first exposure time and then to remain almost constant. The rapid initial weight loss of PLA_{100}-0 can be related to its high degradability and the high enzymatic activity during the first hours. It should be recalled that the buffer solution was changed after 30 h and then after 54 h. One can note that, for the 0−30 h period, the degradation rate of PLA_{100}-0 was the highest during the first 6 h; it decreased in the 6−18 h period and decreased further in the 18−30 h period, which can be assigned to the decrease of enzymatic activity. In contrast, in the case of PLA_{50}, the decrease of degradation rate was not observed in the same periods. It could be assumed that water absorption and swelling, which favored the enzymatic attack, compensated the decrease of enzymatic activity, as will be shown in the following (Figure 3). Similar observations were made for the periods of 30−54 and 54−78 h.

It is noteworthy that in the control medium (without enzyme) weight loss was negligible in all cases (less than 0.4% after 78 h), which clearly indicated the strong catalytic effect of proteinase K on the degradation of PLA polymers.

Water uptake data of PLA_{100}-0 and PLA_{50} are shown in Figure 3. PLA_{50} appeared much more hydrophilic than PLA_{100}-0. During the first 30 h, the water uptake ratio of PLA_{50} increased continuously to attain 9%. From 30 to 54 h, an acceleration of water uptake up to 23% was detected. Beyond, the water uptake ratio remained almost constant, probably because of saturation. In contrast, PLA_{100}-0 appeared very hydrophobic with less

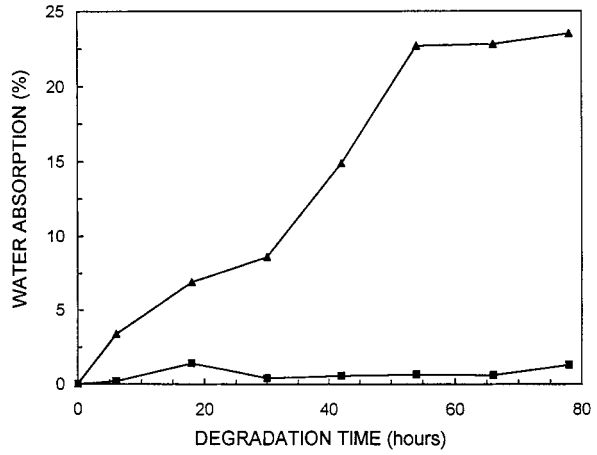

Figure 3. Water absorption changes of PLA_{100}-0 (■) and PLA_{50} (▲) films during the enzymatic degradation at 37 °C.

than 2% of absorbed water during the degradation period. All the other PLA_{100} samples exhibited similar low water contents.

SEC was used to follow the changes of molecular weights and polydispersity during the enzymatic degradation. No significant changes were detected in either case. This is in agreement with the characteristics of enzymatic degradation, i.e., weight loss without molecular weight decrease.

Discussion

The results presented above confirmed that proteinase K is able to strongly accelerate the degradation of PLA polymers. Weight loss data clearly showed that, below a certain crystallinity (about 26%), PLA_{100} exhibits actually the same enzymatic degradability because the great majority of material is amorphous and accessible for enzymatic attack. Above that crystallinity, the degradation rate decreases with increase of crystallinity (Table 1). This is in agreement with literature data.[14]

Another factor to be considered is the water uptake ratio or hydrophilicity of the polymers. It is worth noting that this factor has not yet been mentioned in the literature. In contrast to what was expected, PLA_{50} degraded only slightly less rapidly than PLA_{100}. This finding could be assigned to the much higher water uptake ratio of the former. It is likely that the large amounts of absorbed water led to the swelling of PLA_{50} samples and facilitated the enzymatic attack. Degradation of PLA_{50} was thus enhanced as compared with PLA_{100}, which absorbed very little. In the case of PLA_{100}-0, however, the presence of 100% of L-lactyl units made it intrinsically much more degradable than PLA_{50}. The effect of stereochemistry was more important than that of water uptake. Therefore, the overall degradation rate of PLA_{100}-0 was slightly higher than that of PLA_{50}.

The fact that unannealed PLA_{100} was much more hydrophobic than PLA_{50} could be related to the configurational structures. With 100% of L-lactyl units, PLA_{100} chains should be more densely packed and less mobile than PLA_{50} ones, in agreement with the fact that T_g of PLA_{100} was higher by 5 deg than that of PLA_{50}. As a consequence, water can more easily penetrate PLA_{50} material. Similar behavior was previously observed during the hydrolytic degradation of these polymers.[6,7]

It is also of interest to note that PLA_{50} lost 76% of its initial material after 78 h. In other words, at least 26% of D-lactyl units were degraded. Taking into account the fact PLA_0 is not degradable,[12] the distribution of L-lactyl and D-lactyl comonomers along polymer chains should be a very important factor determining the enzymatic degradability of various PLA stereocopolymers.

In the literature, the contradictory data reported by Reeve et al. and MacDonald et al. could be explained by the involvement of many uncontrolled factors.[12,13] Another important factor is the hydrophilicity of the polymers. According to the results shown above, the content of absorbed water greatly determines the enzymatic degradability. This could be one of the sources of some unexpected results obtained by these authors.[12,13]

In conclusion, the enzymatic degradation by proteinase K of PLA stereocopolymers depends on many factors such as crystallinity, stereochemistry, and hydrophilicity. It should be strengthened that the hydrophilicity of the polymers can play an important role in the enzymatic degradation process. Further studies are under way to elucidate the mechanism and characteristics of the enzymatic degradation of various PLA polymers.

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