Enzymatic Degradability of Poly(lactide): Effects of Chain Stereochemistry and Material Crystallinity

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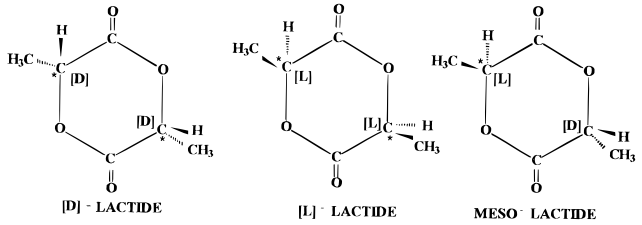
ABSTRACT: Polylactide (PLA) stereocopolymers with (L) repeat unit contents of 75%, 80%, 82%, 85%, 90%, 91%, 92%, 94%, and 95% were prepared from mixtures of (L)-/(D)-lactide and (L)-/*meso*-lactide. Compression molding of these products gave amorphous films which, for (L) contents ≥90%, were also annealed above T_g to crystallize. Analyses by differential scanning calorimetry and wide angle X-ray scattering gave information on the crystalline order of PLA films. For identical (L) contents, stereocopolymers of (L)-/(D)-lactide had higher crystallinities than those from (L)-/*meso*-lactide. PLA films were incubated with proteinase K (from *Tritirachium album*), and the enzyme-catalyzed film weight loss rates were measured. Film crystallinity, chain stereochemical composition, and repeat unit sequence distribution were analyzed as independent variables affecting film enzymatic hydrolysis. Amorphous films from (L)/(D)-lactide copolymerizations with (L) compositions ranging from 80% to 95% exhibited film weight loss rates that were almost identical. Also, amorphous PLA films prepared from (L)-/*meso*-lactide copolymers for (L) contents of 80−95% showed a similar invariability in weight loss rates. It was concluded that proteinase K has a high degree of tolerance for (D) repeat units. Amorphous PLA films from (L)-lactide/*meso*-lactide copolymerizations had weight loss rates which were about 43% slower than amorphous PLA films from (L)-/(D)-lactide copolymerizations. These results were analyzed considering differences in chain stereosequence distributions. Proteinase K showed an extraordinarily high sensitivity to film crystalline order. For example, the decrease in the film weight loss rate due to crystalline order for a 95% (L) (L)-/(D)-lactide stereocopolymer was 93%.

Introduction

Polylactides (PLAs) are known to be biocompatible[1−5] and are hydrodegradable. [6−14] They have found use in materials for controlled release devices [14,15] as well as other medical applications.[4,16−20] In recent years there has been considerable effort to utilize PLAs for plastics which, upon disposal, will be biodegradable.[21,22]

The polymerization of lactide stereoisomers (see structures below) is believed to proceed primarily through a pair addition mechanism.[23,24]



Copolymerization of (L)-/(D)-lactides leads to predominantly isotactic (iso) diad sequences, whereas polymerization of *meso*-lactide introduces syndiotactic (syn) lactic acid diad sequences. Thus, the main chain of PLAs can be designed to have numerous stereochemical variants, which provides a mechanism to regulate corresponding material properties. Polymerization of 100% (L)-lactide ((L)-(L) stereocenters) results in the formation of a semicrystalline (L)-PLA with a melting transition at ∼180 °C and a T_g at ∼67 °C.[25,26] Copolymerizing equal quantities of (L)- and (D)-lactides using non-stereoregulating catalysts[27] results in an amorphous material with a T_g of 58 °C.[25,26] The configurations of lactide monomer chiral centers are retained on conversion to linear polymer chain repeat units when the initiators used are tin salts such as stannous octanoate, tin tetrachloride, or tetraphenyltin.[27−30]

While the hydrolytic degradability of PLA has been studied by a number of laboratories,[5−13] reports on the enzymatic degradability of PLA have been few.[3,5,6,31,32] In a previous study performed in our laboratory, Reeve *et al.*[31] prepared various PLA stereocopolymers from mixtures of (D)- and (L)-lactide using stannous octanoate as the catalyst. Films of these polymers prepared by solution casting were annealed to crystallize and then exposed to proteinase K which is a fungal protease produced by the mold *Tritirachium album*.[33] Proteinase K preferentially degraded (L)- as opposed to (D)-PLA. A decrease in the (L) repeat unit content from 100% to 92% led to a large decrease in crystalline order and corresponding increases in film weight loss rates. However, the experiments performed on semicrystalline PLA films only considered superimposed effects of polymer stereochemistry and material crystallinity on film degradability.[31]

Unlike PLA, the enzymatic degradability of poly(hydroxyalkanoates) as a function of crystalline morphology and stereochemical composition has received considerable attention.[34−46] Of particular relevance to this paper are the effects on biodegradability of polymer repeat unit sequence distribution at equivalent chain enantiopurity. The enzymatic degradability of P3HB stereoisomers having 50% (*R*)-3HB content but differing in stereosequence distribution have been investigated. Studies by Kemnitzer *et al.*[41] and Parandoosh *et al.*[46] showed that predominantly *syn*-P3HB (*syn*-diad content ∼0.67) was readily degraded by the *Penicillium funiculosum* depolymerase whereas atactic P3HB was a poor substrate subsequent to an initial exposure period. Jesudason *et al.*[35] used the depolymerase produced by *Alcaligenes faecalis* to study the enzymatic degradabil-

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ity of 50% (*R*)-P3HB stereoisomers which differed in *iso*-diad content. Films formed from high (∼88%) *iso*-diad content showed little degradation while films from intermediate (63%) *iso*-diad content degraded steadily at a rate less than that found for bacterial P3HB. In contrast, low *iso*-diad (∼48%) 50% (*R*)-P3HB was a poor substrate after initial rapid degradation. P3HB has a T_g below room temperature and is semicrystalline over a wide range of chain isotacticities. Therefore, it is difficult to design studies directed at determining effects of stereochemical composition, stereosequence distribution, and crystallinity as independent parameters on polymer enzymatic degradability.

This paper builds on our previous investigation of PLA degradability by proteinase K.[31] Since large changes in crystalline ordering and enzymatic degradability were expected for PLA with (L) contents from 90% to 95%, detailed investigations over this stereochemical range were performed. For this purpose, stereocopolymers were prepared by copolymerizations of (L)-/(D)-lactide and (L)-/*meso*-lactide. Amorphous films were prepared by compression molding and quenching from the melt. These films were also obtained with crystalline order by annealing above T_g. In addition, PLA stereocopolymers were prepared over the compositional range from 75% to 85% (L). The crystallinity of PLA films was determined by differential scanning calorimetry (DSC) and wide angle X-ray scattering (WAXS). The above series of polymers provided an opportunity to study how film crystalline order, chain stereochemical composition, and repeat unit sequence distribution, taken as independent variables, affected interactions between PLA films and proteinase K. Furthermore, the tolerance of proteinase K for (D)-lactic acid repeat units in PLA chains was investigated.

Experimental Section

(A) Instrumental Methods. Molecular Weight Measurements. All molecular weights were determined by gel permeation chromatography (GPC) utilizing a Waters Model 510 pump, Model 410 differential refractometer, and a Model 717 Plus autosampler with 500-, 10^3-, 10^4-, and 10^5-Å ultrastyragel columns placed in series. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Sample concentrations of ∼0.3% w/v and injection volumes of 100 μL were used. Polystyrene standards with a low dispersity (Polysciences) were used to generate a calibration curve. Data were recorded and manipulated using the Windows based Millenium 2.0 software package.

Thermal Analysis. Differential scanning calorimetry (DSC) studies were conducted on a DuPont DSC 912 equipped with a TA 2000 data station, using between 4 and 10 mg of sample, a heating rate of 10 °C/min, and a nitrogen purge. Data reported for the melting temperature (T_m) and enthalpy of fusion (ΔH_f) were taken from the first heating scan. The reported T_m was the peak melting temperature of the largest endotherm transition. ΔH_f values were taken as the cumulative value over the entire melting transition range.

X-ray Analysis. Wide angle X-ray scattering (WAXS) was performed using a Rigaku 18 kW rotating anode operated at 40 kV/50 mA and Cu Kα radiation (1.5405 Å). A scanning speed of 2°/min with a sample interval of 0.05° was used. Degrees of crystallinity (χ_c) were calculated from diffracted intensity data in the range $2\theta = 10-28°$ according to a method described by Bloembergen *et al.*[47] in that the area of the crystalline peaks were divided by the total area of the crystalline peaks and the amorphous scattering.

Nuclear Magnetic Resonance (NMR). Carbon (^{13}C) NMR spectra were recorded on a Bruker 250 ARX spectrometer at 62.9 MHz. Samples were measured in 5 mm tubes in $CDCl_3$ at a concentration of about 4.0% w/w. Chemical shifts in ppm were referenced relative to chloroform at 77.00 ppm. The following parameters were used for the data acquisitions: temperature 298 K, 32K data points, relaxation delay 2.0 s, pulse width 30°, acquisition time 1.6 s, and 1500 transients.

Polarimetry. Optical rotation measurements were made on a Perkin-Elmer 241 polarimeter attached to a refrigerated constant-temperature circulator. Measurements to determine the specific rotation were recorded at 20 °C in toluene (1.0 g/100 mL) at the sodium D line and are reported as follows: $[\alpha]^{20}_D$ = specific rotation (*c* is concentration in grams per 100 mL of solvent).

(B) Synthetic Procedures. The method used for the bulk copolymerizations of (L)-lactide/(D)-lactide and (L)-lactide/*meso*-lactide followed that previously reported by Reeve *et al.*[31] with modifications described below. The $[\alpha]^{20}_D$ (c = 1.0, toluene) for the (L)- and (D)-lactide (Purac America, used as received) were −277° and +299°, respectively (literature $[\alpha]^{20}_D$ for (L)-lactide in toluene is −285[48]). The method used for purification of *meso*-lactide (donated by Cargill) is summarized as follows. Approximately 20 g of the "as-received" *meso*-lactide (mp 44.7−45.0 °C) was dissolved in 28 mL of dry diethyl ether by heating to reflux. The solution was then cooled slowly to 0 °C, and the crystals were separated from the mother liquor by vacuum filtration, washed with cold dry ether under inert atmosphere, and dried. The product (15 g) melted at 46.4−47 °C which is 3 °C higher than described in the literature for *meso*-lactide with a free acid content of less than 1 mequiv/kg.[49]

The polymerizations were carried out in screw-cap vials with Teflon coated silicone septa disc lined caps. The polymerization vials were silanized (chlorotrimethyl silane), washed with methanol, flame dried, and placed in a desiccator containing P_2O_5 as desiccant to cool. In an argon purged glovebag, the monomers (5 g total) were transferred into polymerization vials which contained magnetic stirbars. The capped vials were then submerged into an oil bath at 130 °C with stirring for a time period (∼2 min) necessary to melt the monomers. The vials were removed from the bath, stannous 2-ethylhexanoate (Sigma, 80 μL for (L)-/(D)-lactide and 8 μL for (L)-/*meso*-lactide copolymerizations) was transferred via syringe under an argon atmosphere into the vials, the vials were hand shaken to ensure mixing, the oil bath temperature was lowered to 120 °C, and the polymerization was carried out at this temperature for 6 h. Polymer products were isolated and purified as previously described[31]. Typically, the yield of polymer from monomer was ∼85%. ^1H NMR spectra of selected synthesized samples were recorded, and the spectra obtained agreed with those previously published for PLA.[50]

(C) Film Preparation. Fibrous PLA products were first dried in a vacuum desiccator for 24 h (0.05 mmHg). Films (about 0.1 mm thickness) were prepared by compression molding using a Dake hydraulic press equipped with heating plates. The polymers were placed between two polished metal plates lined with release sheets (NTI Technologies). The metal plates containing the polymer sample were inserted between the press heating plates; the metal plates were first maintained at 360 °F for 1 min with no applied pressure and then for 1 min with an applied pressure of ca. 255 psi. The processed films were then rapidly quenched by cooling with dry ice to give amorphous materials. To crystallize, films with (L)-contents ≥90% were annealed at 75 °C for 15 h.[51] All films (annealed and not-annealed) were then aged at 30 °C for 24 h to remove residual stress[52,53] and then stored over Drierite at ambient temperature until use.

(D) Biodegradation Studies: Measurements of Film Weight Loss Rates. The method used follows that previously reported[31] with the following modifications. PLA films (1.0 cm × 1.0 cm) with an approximate thickness of 0.1 mm were each placed in separate vials containing 5 mL of Tris-HCl buffer (pH 8.6), 1 mg of proteinase K (Sigma, lyophilized powder, 97% protein),[54] and 1 mg of sodium azide (Fisher). Three replicate films in separate vials were used to determine film weight loss at a specified incubation time. The film−enzyme incubations were carried out at 37 °C in a rotary shaker (250 rpm). At sampling times, the respective films were removed from the shaker incubator, rinsed thoroughly with distilled water, and then dried in vacuo (0.05 mmHg) at room temperature over P_2O_5 until a constant weight was

Table 1. Stereochemical Composition, Molecular Weight, and Film Thickness Values for Synthesized PLA Stereoisomers

sample[a]	mol % in feed (L,L)/(D,D)[b]	mol % in feed (L,L)/(L,D)[c]	M_n[d,e] (g/mol)	film thickness (mm)	M_w/M_n[d]
PLA-95	95/5		71 000 (33 000)	0.12 (±0.01)	1.58
PLA-95M		90/10	nd[f]	0.15 (±0.01)	nd
PLA-94	94/6		61T000 (36 000)	0.13 (±0.01)	1.64
PLA-94M		88/12	nd	0.19 (±0.02)	nd
PLA-92	92/8		70 000 (53 000)	0.11 (±0.01)	1.69
PLA-92M		84/16	225 000 (110 000)	0.15 (±0.02)	1.60
PLA-91	91/9		nd	0.13 (±0.02)	
PLA-90	90/10		64 000 (30 000)	0.12 (±0.02)	1.62
PLA-90M		80/20	187 000 (87 000)	0.17 (±0.02)	1.50
PLA-85	85/15		130 000 (30 000)	0.11 (±0.02)	1.92
PLA-85M		70/30	130 000	0.09 (±0.01)	2.23
PLA-82	82/18		70 000 (32 000)	0.08 (±0.01)	1.91
PLA-82M		64/36	120 000	0.10 (±0.01)	1.85
PLA-80	80/20		70 000 (32 000)	0.10 (±0.02)	1.72
PLA-80M		60/40	70 000 (44 000)	0.09 (±0.01)	1.65
PLA-75	75/25		120 000	0.10 (±0.01)	2.08
PLA-75M		50/50	70 000 (34 000)	0.10 (±0.02)	1.66

[a] Samples denoted with M indicate stereocopolymers prepared with *meso*-lactide [b] Ratio of stereoisomerically pure (L)-lactide to (D)-lactide in monomer feed. [c] Ratio of stereoisomerically pure (L)-lactide to *meso*-lactide in monomer feed. [d] Determined by GPC(see Experimental Section). [e] Values given are for solution precipitated products while the numbers in parentheses are for compression molded amorphous films. [f] Not determined.

achieved. Experimental weight loss values represent averages of determinations from the three replicate films.

Results and Discussion

Polymer Film Characterization. Crystalline Morphology, Molecular Weight, and Repeat Unit Sequence Distribution. PLA products in a range of stereochemical compositions and differing in repeat unit sequence distribution (see Table 1) were synthesized by the ring-opening copolymerization of lactide stereoisomers using stannous octanoate as the catalyst (see Experimental Section). Molecular weights of the solution precipitated products prior to thermal processing were measured by GPC, and the M_n values ranged between 70 000 and 130 000 g/mol for the series of samples with (L) values between 75% and 85%. A few products with (L) values ≥90% prepared using *meso*-lactide had higher M_n values (up to 225 000 g/mol, see Table 1). The formation of films by compression molding resulted in significant molecular weight loss (from 15% to 76%) as is shown in Table 1 (values in parentheses are subsequent to thermal processing). Since the variability in product molecular weights described above was a concern as to its effect on film biodegradability, a study was undertaken using PLA products prepared by copolymerization of (L)-(D)-lactide (90:10) which had M_n values of 30 000, 100 000, and 200 000 g/mol. Interestingly, the normalized weight loss rates for these films of identical stereochemical composition and crystallinity (based on DSC measurements) showed little deviation (4.3, 3.8, and 4.2 $\mu g \cdot mm^{-2} \cdot h^{-1}$, respectively). Thus, it was concluded that, over a wide range of PLA molecular weights, the effects of PLA chain length on proteinase K catalyzed degradation rates are not significant. Furthermore, the lack of a molecular weight effect on polymer degradation suggests that proteinase K has *endo* enzyme activity.

Previous work has documented that PLA, when heated under conditions (temperature, time, catalyst) dissimilar to those used herein for film preparation, may undergo randomization due to transesterification.[23] To determine whether the repeat unit sequence distribution of PLAs was altered due to compression molding, 62.9 MHz ^{13}C NMR analyses (see Experimental Section) were performed on PLA-80 (80% (L) repeat units, copolymerization of (L)- and (D)-lactides) and PLA-80M (80% (L) repeat units, copolymerization of (L)- and *meso*-lactides) before and after compression molding. ^{13}C NMR spectra of PLA stereocopolymers have been reported by others, and assignments of triad and pentad stereosequences were made by observation of the methine and carbonyl carbons.[23,55−57] Comparisons of the relative intensities of triad stereosequences prior and subsequent to thermal processing showed no detectable randomization due to transesterification events (spectra not shown). Furthermore, previous work using stannous octanoate as the catalyst for lactide ring-opening polymerization showed that racemization of the monomer does not occur.[27] Therefore, it was concluded that: (1) thermal processing did not alter product repeat unit sequence distribution, (2) the relative stereosequence fractions of products are predictable based on random propagation statistics,[27,58] and (3) the (L) composition of the products was identical to that of the monomer feed.

All of the films prepared by compression molding followed by rapid quenching from the melt using dry ice were amorphous based on WAXS (no crystalline reflections) and DSC (absence of melting transitions). Furthermore, PLA samples prepared with (L) contents of 85% or less did not crystallize even after elevated temperature annealing (75 °C, 15 h). Thus, a series of amorphous films (no crystallinity effects) were obtained which differed in (L) content and stereosequence distri-

Table 2. Themal Properties and Percent (%) Crystallinity Results for PLA Stereoisomers

sample	heat of fusion (cal/g)[a,c]	T_m (°C)[a,c]	% crystallinity[b,c]
PLA-90	2.3	141	10
PLA-90M	amorphous	amorphous	0
PLA-91	4.5	140	43
PLA-92	5.0	146	44 ± 3[d]
PLA-92M	4.5	133	28
PLA-94	8.1	153	49
PLA-94M	7.8 ± 0.2[d]	141 ± 0.2[d]	44
PLA-95	10.5	156	56
PLA-95M	6.0	142	51

[a] Determined by DSC (see Experimental Section). [b] Percent crystallinity determined by WAXS using the calculation method described by Bloembergen *et al.*[35] [c] Determined after annealing at 75 °C for 15 h and aging at 30 °C for 24 h. [d] Standard deviation calculated based on three replicate samples.

bution. Films from products with >85% (L) were also annealed above T_g (75 °C, 15 h) to investigate effects on degradation caused by crystalline order.

The results of DSC and WAXS analyses on films subsequent to crystallization and aging are shown in Table 2. Increase in the (L) content of PLA products allows the preparation of films with relatively higher crystalline ordering (see Table 2). Interestingly, PLA products of identical (L) contents but synthesized using *meso*-lactide in place of (D)-lactide had relatively lower degrees of crystalline ordering. This is readily apparent from observation of the DSC and WAXS results in Table 2. Thus, the introduction of equivalent (D) units along a polymer chain as crystalline defects within an (L) crystalline lattice by using (D)-(L) or (L)-(D) dyad sequences results in a greater disruption to the material crystalline order than from paired (D)-(D) neighboring groups. This is understandable since, in the later case, the effective crystalline ordering disruption per (D) repeat unit is less because the (D) units are paired.

PLA−Enzyme Incubations. Film Weight Loss Studies. Incubation of PLA films with proteinase K in Tris-HCl buffer (pH 8.6) was carried out (see Experimental Section). By determining weight loss at specific time intervals, curves were generated of the normalized weight loss as a function of exposure time (not shown). The normalized weight loss[59] is the measured weight loss divided by the initial surface area and is given in units of $\mu g/mm^2$. Plots of $\mu g/mm^2$ versus time (not shown) were linear up to film weight loss values of ~60%.[60] The slopes of lines generated from these plots were used to determine the weight loss rates ($\mu g \cdot mm^{-2} \cdot h^{-1}$), which are shown in Figures 1 and 2. Controls run where PLA films were incubated in the absence of enzyme for incubation periods as long as the maximum enzyme exposure times (48 h) showed no measurable weight loss.

Figure 1 shows the weight loss rates for amorphous PLA films prepared from copolymers of (L)-lactide with either (D)- or *meso*-lactide. The compositional range studied was from 75% to 95% (L)-lactic acid repeat units. Surprisingly, amorphous PLA copolymers prepared from (L)- and (D)-lactide show little to no change in weight loss rate for (L) compositions ranging from 80% to 95%. Thus, the stereochemical tolerance of proteinase K for (D) repeat units is unexpectedly high. This broad specificity range can be explained if the binding of proteinase K to amorphous (L)-/(D)-lactide copolymer film surfaces was not substantially altered over this compositional range and assuming that PLA cleavage by proteinase K occurs at intrachain ester sites (*endo*-type mechanism). Thus, assuming that cleavage events

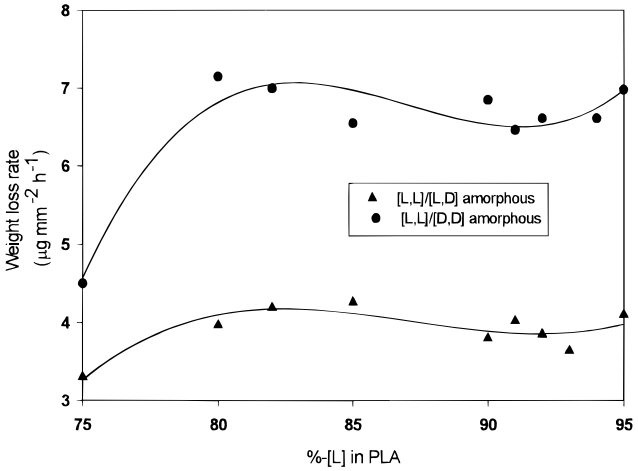

Figure 1. Film weight loss rates ($\mu g \cdot mm^{-2} \cdot h^{-1}$) for amorphous PLA films prepared from (L)-/(D)-lactide and (L)-/*meso*-lactide copolymers having (L) contents from 75% to 95%.

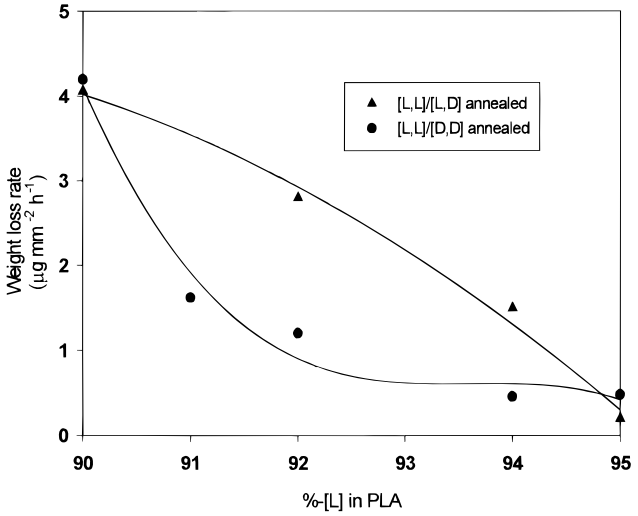

Figure 2. Film weight loss rates ($\mu g \cdot mm^{-2} \cdot h^{-1}$) for annealed PLA films prepared from (L)-/(D)-lactide and (L)-/*meso*-lactide copolymers having (L) contents from 90% to 95%.

occur most rapidly between (L)-(L) units, the concentration of (L)-(L) substrate sequences may saturate proteinase K cleavage sites so that maximum enzyme−substrate conversion rates are observed. In such a case, the enzyme would be the limiting reagent.

Further study of Figure 1 shows that PLA films from products prepared by copolymerizations of (L)- and *meso*-lactides also show a broad stereochemical window (~80−95%-(L) lactic acid repeat units) where the film weight loss rates remain almost unchanged. This result can similarly be rationalized as above for (L)-/(D)-lactide stereocopolymers. For both copolymer sets, the weight loss rates showed apparent decreases for corresponding decreases in (L)-lactic acid content from 80% to 75% (see Figure 1). Thus, the introduction of somewhere between 20% and 25% (D) units resulted in an apparent decrease in proteinase K activity for PLA film degradation (at the enzyme concentration used). Possibly, at concentrations of ~25% (D) repeat units, the enzyme is no longer the limiting reagent due to changes in enzyme−film binding characteristics and/or the decreased concentration of (L)-(L) diads. However, it should be noted that the decrease in weight loss rate between PLA-80M and PLA-75M was much less significant than for PLA-80 to PLA-75. Therefore, the degradation characteristics of these two series of amorphous copolymers between 80% and 75% (L) units are markedly different. Future work

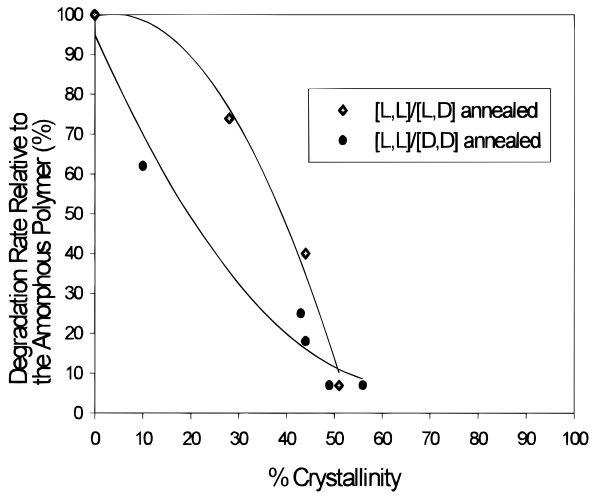

Figure 3. Plot of weight loss rates relative to the amorphous polymer ($rate_{annealed}/rate_{amorphous} \times 100$) for annealed PLA films versus % crystallinity determined by WAXS.

will better define the (D) repeat unit content required to cause large decreases in rate for the (L)/*meso* copolymer series.

Comparison of the film weight loss rates in Figure 1 shows that amorphous (L)-lactide/*meso*-lactide copolymer films with (L) contents between 80% and 95% degraded at a weight loss rate which was ~43% slower than corresponding amorphous (L)-lactide/(D)-lactide copolymer films. These results demonstrate that PLA−enzyme interactions must be dependent on the composition of chain stereosequences. One possible explanation for these results is that (L)-(D)-(L) triad sequences present in (L)-/*meso*-lactide but not (L)-/(D)-lactide copolymers act as an inhibitor and that enzyme−inhibitor interactions are saturated at low inhibitor concentration (for the enzyme concentration used in this work). This explanation is consistent with the observation that an increase in the (D) content from 5% to at least 20% for amorphous (L)-/*meso*-lactide copolymer films did not change film weight loss rates.

The weight loss rates for crystallized PLA films with (L) contents between 90% and 95% prepared by copolymerizations of (L)-/(D)-lactide are shown in Figure 2. A decrease in the weight loss rates for PLA-95, PLA-94, PLA-92, PLA-91, and PLA-90 of 93% (6.9−0.48 $\mu g \cdot mm^{-2} \cdot h^{-1}$), 93% (6.6−0.46 $\mu g \cdot mm^{-2} \cdot h^{-1}$), 82% (6.5−1.2 $\mu g \cdot mm^{-2} \cdot h^{-1}$), 75% (~6.5−1.6 $\mu g \cdot mm^{-2} \cdot h^{-1}$), and 38% (6.8−4.2 $\mu g \cdot mm^{-2} \cdot h^{-1}$), respectively, was observed. As was discussed above, the weight loss rates for PLA-90 to PLA-95 were not affected by the polymer chain (L) content when stereochemistry was analyzed as the independent variable. Thus, assuming crystallinity is the independent parameter which controls the degradation rate, a plot of $rate_{annealed}/rate_{amorphous} \times 100$ versus % crystallinity was constructed (see Figure 3). The decrease in weight loss rates was largest (~14 times) when comparing the semicrystalline and amorphous forms of PLA-94 and PLA-95 due to the relatively high crystalline ordering of these PLA products. It is interesting that even though PLA-94 and PLA-95 have very different ΔH_f and % crystallinity values (see Table 2), they have almost identical weight loss rates (0.46 and 0.48 $\mu g \cdot mm^{-2} \cdot h^{-1}$, see also Figure 3). Possibly, constraints imposed on proteinase K enzyme degradation by crystalline ordering of PLA-94 and PLA-95 are similar. PLA-90 has an χ_c of only 10%, but this low crystallinity still results in a decrease in the weight loss rate by 38% (see Figure 3). Thus, even a low level of film crystallinity causes a substantial constraint on productive PLA−proteinase K interactions which lead to film weight loss.

The weight loss rates for annealed and amorphous films prepared from PLA-90M, PLA-91M, PLA-92M, PLA-93M, and PLA-95M ((L)-/*meso*-lactide copolymers) are shown in Figures 2 and 1, respectively. Although not determined experimentally, it is reasonable to assume by interpolation that the weight loss rate for amorphous PLA-94M is ~3.8 $\mu g\ mm^{-2}\ h^{-1}$. The deceleration in the weight loss rates due to crystalline ordering for PLA-94M and PLA-92M (see Figure 3) was 60% (3.8−1.5 $\mu g \cdot mm^{-2} \cdot h^{-1}$) and 26% (3.8−2.8 $\mu g \cdot mm^{-2} \cdot h^{-1}$), respectively. The differences in weight loss rates were much less than was observed above for (L)-/(D)-lactide copolymers of identical (L) repeat unit content (see above). These results are explained by the fact that annealed (L)-/*meso*-lactide copolymers have poorer crystalline ordering and lower levels of crystallinity than corresponding (L)-/(D)-lactide copolymers (see Table 2). The similarity in weight loss rates of annealed PLA-90 and PLA-90M films results from the fact that the former film is semicrystalline whereas the latter is amorphous but is decelerated by the introduction of *meso*-lactide repeat units. The % crystallinity of PLA-95M is intermediate to that for PLA-94 and PLA-95. Interestingly, the degradation rates of annealed PLA-95M, PLA-94, and PLA-95 relative to their corresponding amorphous films are identical (Figure 3), illustrating how crystallinity is the dominant factor controlling the weight loss rate. However, annealed PLA-95M film has a weight loss rate that is about 50% less than PLA-95 (see Figure 2). Thus, it appears that, at high film crystalline order, stereosequence effects caused by *meso*-lactide repeat units can also influence the weight loss rate. Since Figure 3 attempts to eliminate effects of sequence distribution between the two series of copolymers ((L)-/(D)-lactide and (L)-/*meso*-lactide) by normalizing the degradation rate relative to that of the amorphous polymer, it was anticipated that there would be close agreement between the two curves in Figure 3. Although there are not sufficient data points of similar crystallinity levels between the two copolymer sets, it appears that there was substantial deviation between the curves. This may be explained by other differences in film crystalline morphology which are not taken into account by % crystallinity measurements.

Summary of Results

By preparing both amorphous and semicrystalline films from polymers having variable (L) content, it was possible to consider crystalline order and chain stereochemical composition as independent parameters affecting PLA enzymatic degradability. In addition, comparison of polymers prepared from (L)-/(D)-lactide and (L)-/*meso*-lactide copolymerizations provided information on how chain stereosequence distribution affects PLA enzymatic degradability. Studies on amorphous films from (L)-(D)-lactide as well as (L)-*meso*-lactide copolymerizations showed a remarkable independence of film weight loss rates on (L) content for compositions ranging from 80% to 95%. These results show that proteinase K has a high degree of tolerance for (D) repeat units. However, amorphous PLA films from (L)-lactide/*meso*-lactide copolymerizations had weight loss rates which were about 43% slower than amorphous PLA films from (L)-lactide/(D)-lactide copolymerizations. These results demonstrate how polymer repeat unit sequence distribution, taken as an independent variable, can dramatically affect enzyme catalyzed polymer degrada-

tion. Furthermore, the deceleration of proteinase K catalyzed PLA degradation caused by crystallinity effects was determined. Proteinase K showed an extraordinarily high sensitivity to film crystalline order. For example, the decrease in the film weight loss rate due to crystalline order for PLA-95 was 93%. Furthermore, PLA-90 which has an χ_c of only 10% had a decrease in weight loss rate by 38% due to film crystallinity. The results of this study raise a number of questions that we plan to address in future work. For example, to better understand the PLA−proteinase K system and test some of the hypotheses presented herein, work has been initiated using purified proteinase K enzyme preparations to investigate the following: (i) a larger range of PLA stereochemical compositions, (ii) concentration of enzyme at PLA surfaces, (iii) reaction kinetics as a function of enzyme and surface area concentrations, and (iv) PLA single crystal substrates as model systems.

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References and Notes

(1) Vert, M.; Li, S.; Garreau, H. *Clin. Mater.* **1992**, *10*, 3.
(2) Therin, M.; Christel, P.; Li, S.; Garreau, H.; Vert, M. *Biomaterials* **1992**, *13* (9), 594.
(3) Makino, K.; Arakawa, M.; Kondo, T. *Chem. Pharm. Bull.* **1985**, *33* (3), 1195.
(4) Pennings, J. P.; Dijkstra, H.; Pennings, A. J. *Polymer* **1993**, *34* (5), 942.
(5) Fukuzaki, H.; Yoshida, M.; Asano, M.; Kumakura, M. *Eur. Polym. J.* **1989**, *25* (10), 1019.
(6) Vert, M.; Li, S. M.; Spenlehauer, G.; Guerin, P. *J. Mater. Sci.: Mater. Med.* **1992**, *3*, 432.
(7) Leenslag, J. W.; Pennings, A. J.; Bos, R. M.; Rozema, F. R.; Boering, G. *Biomaterials* **1987**, *8*, 311.
(8) Miller, R. A.; Brady, J. M.; Cutright, D. E. *J. Biomed. Mater. Res.* **1977**, *11*, 711.
(9) Vert, M.; Chabot, F.; Leyray, J.; Christel, P. *Makromol. Chem. Suppl.* **1981**, *5*, 30.
(10) Chu, C. C. *J. Appl. Polym. Sci.* **1981**, *26*, 1727.
(11) Li, S.; Garreau, H.; Vert, M. *J. Mater. Sci.: Mater. Med.* **1990**, *1*, 198.
(12) Li, S.; Vert, M. *Macromolecules* **1994**, *27*, 3107.
(13) Vert, M.; Li, S.; Garreau, H. *J. Controlled Release* **1991**, *16*, 15.
(14) Heller, J. *Biomaterials* **1980**, *1*, 51.
(15) Jakanicz, T. M.; Nash, H. A.; Wise, D. L.; Gregory, J. B. *Contraception* **1973**, *8*, 227.
(16) Vainionpaa, S.; Rokkanen, P.; Tormala, P. *Prog. Polym. Sci.* **1989**, *14*, 679.
(17) Vert, M.; Christel, P.; Chabot, F.; Leray, J. In *Macromolecular Biomaterials*; Hastings, G. W., Ducheyne, P., Eds.; CRC Press: Boca Raton, FL, 1984; pp 120−142.
(18) Leenslag, J. W.; Pennings, A. J.; Bos, R. M.; Rozema, F. R.; Boering, G. *Biomaterials* **1987**, *8*, 311.
(19) Bos, R. R. M.; Rozema, F. R. ; Nijenhuis, A. J. ; Pennings, A. J. ; Jansen, H. W. B. *Br. J. Oral Maxillofac. Surg.* **1989**, *27*, 467
(20) Bos, R. R. M.; Boering, G.; Rozema, F. R.; Nijenhuis, A. J.; Pennings, A. J.; Verway, A. B. *Int. J. Oral Maxillofac. Surg.* **1989**, *18*, 365.
(21) Kawashisma, N.; Ajioka, M.; Suzuki, K. Japanese Patent, Oct. 25, 1994, 06,298,236.
(22) Kimura, K.; Ito, T.; Aoyama, T. Japanese Patent, Sept. 13, 1994, 06,256,480.
(23) Chabot, F.; Vert, M.; Chapelle, S.; Granger, P. *Polymer* **1983**, *23*, 53.
(24) Dittrich, V. W.: Schulz, R. C. *Angew. Makromol. Chem.* **1975**, *176*, 1901.
(25) Zhang, X.; Goosen, M.; Wyss, U. P; Pichora, D. *J. Macromol. Sci., Rev. Macromol. Chem. Phys.* **1993**, *c33* (1), 81.
(26) Lavallee, C. *Proc. Int. Symp. Adv. Polym. Synth.* **1985**, *Aug 26−31*, 441−446.
(27) Kricheldorf, H. R.; Boettcher, C.; Tönnes, K. *Polymer* **1992**, *13* (33), 2817.
(28) Kricheldorf, H. R.; Serra, A. *Polym. Bull.* **1985**, *14*, 497.
(29) Schindler, A.; Harper, D. *Polym. Lett. Ed.* **1976**, *14*, 729.
(30) Schindler, A.; Gaetano, K. D. *J. Polym. Sci., Part C: Polym. Lett.* **1988**, *1* (26) 47.
(31) Reeve, M.; McCarthy, S. P.; Downey, M. J.; Gross, R. A. *Macromolecules* **1994**, *27*, 825.
(32) Williams, D. F. *Eng. Med.* **1981**, *10* (1), 5.
(33) Ebeling, W.; Hennrich, N.; Klockow, M.; Metz, H.; Orth, H. D.; Lang, H. *Eur. J. Biochem.* **1974**, *47*, 91.
(34) Abe, H.; Matsubara, D.; Doi, Y.; Hori, Y.; Yamaguchi, A. *Macromolecules* **1994**, *27*, 6018.
(35) Jesudason, J. J.; Marchessault, R. H.; Saito, T. *J. Environ. Polym. Degrad.* **1993**, *2* (1), 89.
(36) Nishida, H..; Tokiwa, Y. *J. Environ. Polym. Degrad.* **1993**, *1* (1),65.
(37) Kumagai, Y.; Kanesawa, Y.; Doi, Y. *Makromol. Chem.* **1992**, *193*, 53.
(38) Kumagai, Y.; Doi, Y. *Polym. Degrad. Stab.* **1992**, *36*, 241.
(39) Kumagai, Y.; Doi, Y. *Makromol. Chem., Rapid Commun.* **1992**, *13*, 179.
(40) Yamada, K.; Mukai, K.; Doi, Y. *Int. J. Biol. Macromol.* **1993**, *15*, 215.
(41) Kemnitzer, J. E.; McCarthy, S. P.; Gross, R. A. *Macromolecules* **1992**, *22* (25), 5927.
(42) Hideki, A.; Matsubara, I.; Doi, Y.; Hori, Y.; Yamaguchi, A. *Macromolecules* **1994**, *27*, 6018.
(43) Shirakura, Y.; Fukui, T.; Saito, T.; Okamota, Y.; Narikawa, T.; Koide, K.; Tomita, K.; Takemasa, T.; Masamune, S. *Biochim. Biophys. Acta* **1986**, *880*, 46.
(44) Shimamura, E.; Kasuya, K.; Kobayashi, G.; Shiotani, T.; Shima, Y.; Doi, Y. *Macromolecules* **1994**, *27*, 878.
(45) Abe, H.; Matsubara, D.; Doi, Y.; Hori, Y.; Yamaguchi, A. *Macromolecules* **1994**, *27*, 878.
(46) Parandoosh, S.; Jackson, R.; Farrell, R. E.; McCarthy, S. P.; Eberiel, D. T.; Gross, R. A. *Polym. Prepr. Am. Chem. Soc., Div. Polym. Sci.* **1995**, *36* (1), 424.
(47) Bloembergen, S.; Holden, D. A.; Hamer, G. K.; Bluhm, T. L.; Marchessault, R. H. *Macromolecules* **1986**, *19*, 2865.
(48) Aldrich Catalogue of Fine Chemicals.
(49) Nieuwenhuis *et al.* (1991), U.S. Patent No. 5,053,485.
(50) Kricheldorf, H. R.; Berl, M.; Scharnagl, N. *Macromolecules* **1988**, *21*, 286.
(51) The annealing conditions were based on previous work performed in our laboratory (see ref 31 above) and from unpublished results that showed little change in the % crystallinity of PLA polymer films which were annealed at 75 °C for time periods between 4 and 36 h.
(52) Film aging was carried out, since aged and deaged PLA samples degrade at different rates when exposed to proteinase K. Specifically, deaged PLA samples where there is a densification of the amorphous phase degrade at relatively slower rates than aged samples. See ref 41 above.
(53) Cai, H.; Dave, V.; Gross, R. A.; McCarthy, S. P. *Polym. Prepr., Am. Chem. Soc., Div. Polym. Sci.* **1995,** *36* (1), 422.
(54) Proteinase K had 13.8 units/mg of solid and 14.3 units/mg of protein (Biuret) based on values reported by the supplier. One unit will hydrolyze casein to produce color equivalent to 1 μmol (181 μg) of tyrosine/min at pH 7.5 and 37 °C (color by Folin−Ciocalteu reagent).
(55) Lillie, E.; Schulz, R. C. *Makromol. Chem.* **1975**, *176*, 1901.
(56) Schindler, A., Gaetano, K. D. *J. Polym. Sci., Part C: Chem Lett.* **1988**, *26*, 47.
(57) Kricheldorf, H. R.; Boettcher, C.; Tonnes, K. U. *Polymer* **1992**, *33* (13), 2817.
(58) Bovey, F. A. *Chain Statistics and Conformation of Macromolecules;* Academic Press: New York, 1982.
(59) Gu, J.; Eberiel, D. T.; McCarthy, S. P.; Gross, R. A. *J. Environ. Degrad.* **1993** *1* (2), 143.
(60) Linear regression analyses of film weight loss (to <60%) vs time plots were carried out to determine values of slopes shown in Figures 1−3. Correlation coefficients from these analyses are as follows: PLA-75 (0.98); PLA-75M (1.0); PLA-80 (0.99); PLA-80M (0.93); PLA-82 (0.96); PLA-82M (0.98); PLA-85 (0.97); PLA-85M (0.99); PLA-90 annealed (0.99); PLA-90M annealed (0.99); PLA-90 amorphous (0.96); PLA-90M amorphous (0.98); PLA-91M amorphous (0.99); PLA-92 annealed (0.93); PLA-92M annealed (0.99); PLA-92 amorphous (0.98); PLA-92M amorphous (0.99); PLA-94 annealed (0.94); PLA-94M annealed (0.99); PLA-94 amorphous (0.98); PLA-93M amorphous (0.98); PLA-95 annealed (01.0); PLA-95 amorphous (1.0).

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