Polylactide Stereochemistry: Effect on Enzymatic Degradability

Michael S. Reeve,[†] Stephen P. McCarthy,[‡] Milton J. Downey,[§] and Richard A. Gross*,[†]

Polymer Biodegradation Research Center, University of Massachusetts, Lowell, One University Avenue, Lowell, Massachusetts 01854

Received September 14, 1993; Revised Manuscript Received November 3, 1993[⊕]

ABSTRACT: Polylactide (PLA) with % L repeat unit contents of 100 (PLA-100), 99, 96, 94, 92, 85, 80, 75, 70, 50, and 0 were prepared from mixtures of (D)- and (L)-lactide using stannous octanoate as catalyst. PLA films of these samples were prepared by solution casting and then annealing at 75 °C for 36 h to crystallize. Large changes in the crystalline order for PLA with % L compositions between 85 and 100% were observed by differential scanning calorimetry and wide-angle X-ray scattering measurements. The PLA films were incubated with proteinase K (from *Tritirachium album*) to determine enzyme-catalyzed rates of both film weight loss and initial surface degradation. The latter rate values were obtained by monitoring pH changes. Proteinase K preferentially degraded (L)-PLA as opposed to (D)-PLA. Optimal rates for both film weight loss and initial surface degradation were found for PLA-92. In addition, a large increase in the film weight loss rate for PLA-92 relative to both PLA-85 and PLA-94 indicates that a critical disruption of the crystalline order resulted from the introduction of 8% D repeat units. Comparison of degradation rates for PLA-100 at percent crystallinities of 57 and 27% showed large increases in both film weight loss and initial surface degradation rates for the lower crystallinity sample. Initial surface degradation rates were unexpectedly high for PLA-75 and PLA-50 relative to PLA-92. In contrast, a rapid decline in film weight loss rate values was found for PLA stereochemical compositions from 92 to 50% L. However, qualitatively, the rates of initial surface degradation and film weight loss measurements are in agreement. Residual PLA-100 from enzyme incubations where film weight loss exceeded 25% showed little change in molecular weight and increased ΔH_f values as was expected for an enzyme-catalyzed film degradation process at the film surface which occurs preferentially at amorphous domains.

Introduction

Polylactides (PLA's) have been investigated as materials for controlled-release devices,[1,3] degradable sutures,[4,5] absorbable fibers,[6] and implants for bone fixation.[7–10] Currently, there is increasing interest in using PLA for disposable degradable plastic articles.[11] High molecular weight PLA is normally obtained by the ring-opening polymerization of lactide monomer.[12–15] Since the lactide monomer is chiral, the control of PLA stereochemistry is easily achieved by the polymerization of (L)-, (D)-, and *meso*-lactide stereochemical forms. Modulation of the polymer stereochemistry leads to PLA with dramatically different properties. For example, poly[(L)-lactide] [(L)-PLA] is a semicrystalline polymer (T_g at 67 °C, melting transition at 180 °C), while poly(DL-lactide) [(DL)-PLA] is an amorphous material (T_g at 58 °C).[16,17] Lactide ring-opening polymerization may be carried out using a range of catalysts.[13–15,18–24] The catalyst stannous octanoate was used in this work for the bulk polymerization of lactide since the polymerizations can be carried out at relatively low temperatures (120 °C),[13] transesterification reactions are virtually nonexistent,[13] and retention of stereochemical purity during the conversion of monomer to polymer is exceptional (99%).[13]

Although the hydrolytic degradability of PLA has been extensively investigated,[8,25–28] reports of PLA enzymatic degradability have been few. Fukuzaki and co-workers[27] exposed PLA stereocopolymers (% L ranging from 8 to 98) of relatively low molecular weight (M_n ~2000) to several esterase enzymes. The researchers found that PLA hydrolytic degradation was accelerated in the presence of specific enzymes. The lipase from *Rhizophus delemer* was found to have the greatest activity for PLA degradation relative to hog pancreas lipase, carboxylic esterase from porcine liver, and wheat germ lipase. Interestingly, the PLA stereocopolymer which showed the most rapid enzymatic degradation of the series of stereocopolymers studied was that which contained 50% L. Williams[29] reported that L-PLA with a relative viscosity 1.16 (supplied by ICI) was degraded by the enzymes pronase, proteinase K, and bromelain. In this work, (L)-PLA in the form of fibrous particles showed extensive weight loss after enzyme exposures. Makino et al.[30] showed that upon the addition of a carboxylic esterase an accelerated decrease in M_w for (DL)-PLA was observed.

Studies by Kemnitzer et al.[31] in our laboratory were carried out on the enzymatic degradability of poly(β-hydroxybutyrate) (PHB) stereoisomers by the PHB depolymerase from *Penicillium funiculosum*. The relative rates of initial surface enzymatic degradation of the PHB stereoisomer films were measured by monitoring the pH change of the solution as a function of time. Dramatic effects of polymer stereochemistry and the resulting film crystalline morphology on the measured rates of initial surface enzymatic degradation were reported.

The studies described herein use the enzyme proteinase K to study PLA enzymatic degradability. Proteinase K is a fungal protease produced by the mold *Tritirachium album*.[32] *Tritirachium album* was isolated from soil manured with horn chips.[32] Previous characterization of proteinase K reported that the enzyme has a molecular weight estimated by gel filtration of 18 500 ± 500, isoelectric point of 8.9, and a pH optimum activity range between 7.5 and 12.0.[32] Work in our laboratory using PLA-94 as the substrate to determine the pH optimum range showed that the enzyme activity remained constant over the pH range from 9.0 to 8.0 but showed significantly decreased activity at pH 7.5.[33]

* To whom correspondence should be addressed.
† Department of Chemistry.
‡ Department of Plastics Engineering.
§ Advanced Materials Center.
⊕ Abstract published in *Advance ACS Abstracts,* January 1, 1994.

0024-9297/94/2227-0825$04.50/0

In this paper, PLA stereoisomers containing varied % L contents were prepared by the stannous octanoate catalyzed polymerization of (L)-lactide, (D)-lactide, and mixtures of the two monomers. PLA stereoisomer films were then prepared by solution casting. The crystalline order of these films was analyzed by differential scanning calorimetry (DSC) and wide-angle X-ray diffraction (WAXS). The rate of enzyme-catalyzed film weight loss and initial surface degradation was then evaluated using the PLA-degrading enzyme proteinase K. In addition, the effects of the enzyme–substrate exposures on the recovered residual polymer films were analyzed by gel permeation chromatography (GPC) and DSC.

Experimental Section

Instrumental Methods. Molecular Weight Measurements. Molecular weights and dispersities were determined by gel permeation chromatography (GPC). GPC studies were carried out using a Waters Model 510 pump, Model 410 refractive index detector, and Model 730 data module 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 0.5% (w/v) and injection volumes of 100 μL were used. Polystyrene standards with low polydispersities (Aldrich) were used to generate a calibration curve.

Thermal Analysis. Differential scanning calorimetry (DSC) studies were conducted on a DuPont DSC 2910 equipped with a TA 2000 data station, using between 5- and 10-mg sample weights, a heating rate of 10 °C/min, and a nitrogen purge. Melting points are determined by the highest peak melting transition in all cases.

X-ray Analysis. Wide-angle X-ray scattering (WAXS) was performed using a Norelco vertical diffractometer with Bragg–Brentano focusing geometry, diffracted beam monochromator (3-kW constant-potential generator), and Cu Kα radiation. All film samples were mounted on a zero-background substrate. Initial diffraction patterns were collected using a counting rate of 500 counts/s at a power setting of 40 kV and 20 mA, while the final scans were collected using a counting rate of 1000 counts/s at a power setting of 22 kV and 12 mA to keep all reflections on scale. Degrees of crystallinity (X_c) were calculated from diffracted intensity data in the range 2θ = 10–28° according to a method described by Bloembergen.[34] X_c is determined by dividing the area of the crystalline peaks by the area of the crystalline peaks and amorphous scattering.

Nuclear Magnetic Resonance (NMR). Proton (^{1}H) NMR spectra were recorded on a Bruker WP-270 SY spectrometer at 270 MHz. ^{1}H NMR chemical shifts in parts per million (ppm) are reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. The parameters for the polymer spectra are as follows: 3.5% (wt/wt) polymer in $CDCl_3$, temperature 308 K, pulse width 4.9 ms, 32K data points, relaxation delay 0.50 s, 100–200 transients. Peak areas were determined by spectrometer integration.

Infrared Spectra (IR). Spectra were recorded on polymer films cast from chloroform solution onto NaCl plates using a Bruker IFS 113v FT-IR at 25 °C.

Synthetic Procedures. The bulk polymerizations of (L)-lactide, (D)-lactide, and mixtures of these two monomers were carried out using a modification of literature methods.[13,15] Stannous 2-ethylhexanoate, which is also referred to as stannous octanoate (Sigma), and polymerization grade (L)- and (D)-lactide (Purac America and CCA Biochem, both white crystalline powders) were used as received.

The polymerizations were carried out in ampules (25-mL internal volume) which were silanized with chlorotrimethylsilane, washed with methanol, and oven-dried. The monomers, glassware, septa, stirbars, and a scale were placed into a large glovebag (Aldrich) fit with an argon inlet. Weighing and transfer of monomer into the polymerization ampules were carried out within the glovebag. The argon used (99.998%) to purge the glovebag was first passed through a column containing anhydrous phosphorus pentoxide coated silica (obtained from Fluka, column dimensions 50 × 4.5 cm) and then through a column containing Drierite (obtained from VWR, column dimensions 80 × 5 cm) to establish a dry and inert atmosphere within the glovebag. The desired lactide monomer composition (5-g total) was transferred into the polymerization ampules which contained magnetic stirbars, and ampules were capped with rubber septa. The polymerization ampules containing monomer were removed from the glovebag and placed into a silicone oil bath at 120 °C to melt the monomer, and then 0.10 g (2% by weight) of stannous octanoate was transferred via syringe under an argon atmosphere into each ampule. The ampules were then hand shaken to ensure mixing, maintained at 120 °C with magnetic stirring for a total polymerization time of 6 h, and then placed at −15 °C prior to polymer isolation and purification.

PLA products were purified by dissolving the polymerization ampule contents in chloroform (100 mL), removing undissolved materials by filtration, and precipitating the polymer by adding the chloroform polymer solution to a 10 volume excess of cold methanol. The resulting white solids were then redissolved in a minimum volume of chloroform and reprecipitated as above. The PLA products thus obtained were then placed in vacuo 0.005 mmHg) for 48 h at room temperature over P_2O_5 and then stored over Drierite desiccant at −15 °C until use. Typically, the yield of polymer from monomer was ca. 85%.

The ^{1}H NMR and IR of the synthesized samples were recorded, and the spectra obtained agreed with those previously published for PLA.[14,22,35] Results of GPC and DSC analyses of the synthesized products are reported in the Results and Discussion.

Sample Preparation. Thin films of the PLA stereoisomers were prepared by casting from chloroform solution (9% w/v) onto a glass plate covered with Teflon protective overlay (Cole-Parmer). A Gardner knife (set at 35 units) was then passed slowly over the solution to ensure that films of relatively uniform thickness were obtained. Solvent evaporation was first carried out at ambient temperature and pressure over 48 h and then in vacuo (0.005 mmHg) for 48 h at room temperature over P_2O_5. Unless otherwise specified, the films were then annealed above their glass temperature (T_g) values at 75 °C for 36 h in a vacuum oven over P_2O_5 as desiccant. The polymer films were then stored over Drierite at −15 °C until use.

Degradation Studies. Film Weight Loss Studies. Weight loss studies on PLA films were carried out by a modification of existing literature methods.[29,32] A PLA film (2.5 × 1.0 cm) with an approximate thickness of 0.06 mm was placed in a vial containing 5 mL of Tris-HCl buffer (pH 8.6), 1.0 mg of proteinase K (Sigma, lyophilized powder, 80% protein[36]), and 1.0 mg of sodium azide (Fisher, purified). The water used to prepare the above solutions was purified by a Barnstead NANOpure water purification system. For a given experiment, three replicate films in separate vials were used for weight loss measurements at a specified incubation time. The film/enzyme incubations were carried out at 37 °C in a rotary shaker (100 rpm). The buffer/enzyme solution was replaced every 30 h to ensure that enzyme activity remained at a desired level throughout the experiment duration and that the solution pH did not drop below pH 8.0. At sampling times, three vials each containing a polymer film sample type were removed randomly from the shaker incubator, and the films were washed extensively with distilled water and then dried in vacuo (0.005 mmHg) for 48 h at room temperature over P_2O_5 (anhydrous powder).

Initial Surface Degradation Rate Measurements. These studies were carried out on PLA films (0.75 × 0.75 cm) in a cylindrically shaped flask fitted with a side arm (for an argon inlet) and an entrance port through which a pH probe was placed. The procedure followed was a modification of that previously reported by our laboratory.[31] A small stirbar, 2 mL of degassed purified water (see above), and 80 μL of a 5 mg/mL aqueous solution of proteinase K (Sigma; see above) prepared using degassed purified water were introduced into the reaction flask. The enzyme solution was used within 9 h of its preparation and was stored at 5 °C during this time period. This protocol for storage of the enzyme solution was adopted since the activity of the enzyme toward PLA was found to be unchanged for up to 9 h at 5 °C but significantly decreased after 16 h at 5 °C. The pH within the reaction flask was adjusted to 9.0 with a dilute NaOH solution, and then the flask was placed into a water bath at 37 °C for 30 min. After this equilibration time period, the pH

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

sample	mol % in feed (L/D)[a]	% yield[b]	M_n[c]	film thickness (mm)	M_w/M_n[c]
PLA-100	100/0	87	198 000	0.052 (±0.002)	1.72
PLA-99	99/1	83	255 000	0.059 (±0.002)	1.75
PLA-96	96/4	88	245 000	0.058 (±0.003)	1.81
PLA-94	94/6	88	244 000	0.067 (±0.005)	1.78
PLA-92	92/8	86	219 000	0.054 (±0.001)	1.76
PLA-85	85/15	94	169 000	0.086 (±0.003)	2.01
PLA-80	80/20	99	157 000	0.090 (±0.004)	1.95
PLA-75	75/25	93	99 000	0.087 (±0.004)	1.85
PLA-70	70/30	99	103 000	0.087 (±0.004)	1.92
PLA-50	50/50	95	176 000	0.090 (±0.004)	1.93
PLA-0	0/100	82	235 000	0.064 (±0.004)	1.75
PLA-100U[d]	100/0	68	205 000	0.105 (±0.005)	1.59

[a] Ratio of stereoisomerically pure (L)-lactide to (D)-lactide in monomer feed. [b] Methanol-insoluble product. [c] Determined via GPC (see Experimental Section). [d] 100% (L)-PLA solution cast at room temperature without subsequent elevated temperature annealing.

was again adjusted to 9.0 (if needed) and a single film was introduced. Changes in pH as a function of time which correspond to events of chain cleavage were measured continuously by a pH meter attached to a strip chart recorder. pH changes on the strip chart recorder were calibrated using a series of pH standards. The measurements were terminated once the pH decreased to 8.0 so as to stay within the pH optimum range of the enzyme (see Introduction and ref 33). For each sample studied, this procedure was repeated three times, and the values reported are an average of those from the three runs.

Results and Discussion

Polymer Film Characterization. Crystalline Morphology and Molecular Weight Analysis. The synthesis of PLA samples in a range of stereochemical compositions was carried out as was described above (see Experimental Section) using stannous octanoate as catalyst for lactide ring-opening polymerization. It is assumed from previous work that the PLA stereocopolymers thus formed approximate a random stereosequence distribution based on a pair addition mechanism and that the stereochemical compositions of the polymers are identical to that used in the monomer feed.[37] PLA films of these samples were prepared by solution casting from chloroform (see Experimental Section). The film samples were annealed at 75 °C for 36 h (see Experimental Section) to crystallize. It was not established in this work what annealing conditions are required to obtain optimal percent crystallinity values for the respective PLA stereoisomers.

Table 1 shows the mole percent of the respective (D)- and (L)-lactide monomers used in the polymerization reactions and the number-average molecular weight (M_n) and polydispersity (weight average (M_w)/M_n) values measured by GPC of the corresponding polymer products after film annealing at 75 °C for 36 h. Most of the samples had similar molecular weight values, falling within the range M_n = 155 000–245 000. Samples which deviate from this molecular weight range include PLA-99, PLA-75, and PLA-70, which have M_n values of 255 000, 99 000, and 103 000, respectively. The effect of PLA molecular weight (taken as an independent variable) on the enzymatic film weight loss and initial surface degradation rates were not studied as part of this work. It is well known that polymers of relatively lower molecular weight may show accelerated rates of enzymatic degradation because of, for example, the concentration of accessible chain end groups.[38,39] Although efforts were made in this work to minimize differences between the PLA sample molecular weight values, discussion of the results presented below takes into account that an accelerated degradation rate value might result for samples of comparatively lower molecular weight values.

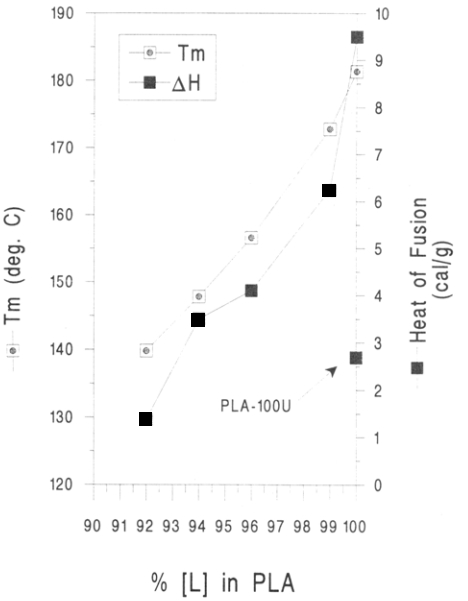

Figure 1. Melting points (T_m) and heats of fusion (ΔH_f) determined by DSC for PLA stereoisomers of variable stereochemical composition.

Table 2. Crystallinity Values Determined by WAXS for Selected PLA Film Samples

film sample	% crystallinity[a]
PLA-100	57 ± 5[c]
PLA-94	44
PLA-92	32
PLA-100U[b]	27

[a] Percent crystallinity was calculated by the method described by Bloembergen et al.[34] [b] 100% (L)-PLA solvent cast at room temperature without subsequent elevated temperature annealing. [c] Standard deviation calculation based on three replicate samples.

DSC analyses on the annealed film samples were performed, and the measured peak melting points (T_m) and heat of fusion (ΔH_f) values are shown in Figure 1 for PLA samples with stereochemical compositions ranging from 100 to 92% L. As the % L repeat units in the polymer is decreased from 100 to 92%, the T_m and ΔH_f values decrease from ca. 180 to 138 °C and 9.5 to 1.4 cal/g, respectively.

Further analysis of the crystalline order present in selected PLA films was obtained by measuring the percent crystallinity by WAXS (see Experimental Section and Table 2). It was determined that PLA-100, -94, -92, and -50 samples had percent crystallinity values of 57, 44, 32, and 0%, respectively. Previous literature on the percent crystallinity of (L)-PLA which had been annealed at 110 °C (14 h) and 130 °C (2 h) reported values of 58[40] and 73%[41] crystallinity, respectively, supportive of the 57% value measured in this work for 75 °C (36 h) annealed (L)-PLA films.

Based on the DSC and WAXS analyses over the compositional range between PLA-100 and PLA-92, only an 8% change in the repeat unit stereochemical composition is needed to cause dramatic changes in the crystalline order of PLA samples. This then creates an opportunity to investigate the enzymatic degradability of PLA stereoisomers which have only small changes in stereochemical composition but large changes in crystalline order. Also, since solution-cast 100% L samples not subjected to elevated temperature annealing (denoted as PLA-100U,

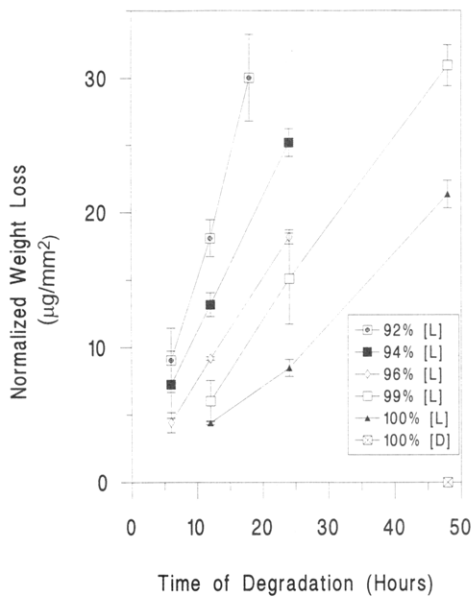

Figure 2. Normalized weight loss ($\mu g/mm^2$) as a function of time for PLA films of variable stereochemical composition exposed to proteinase K.

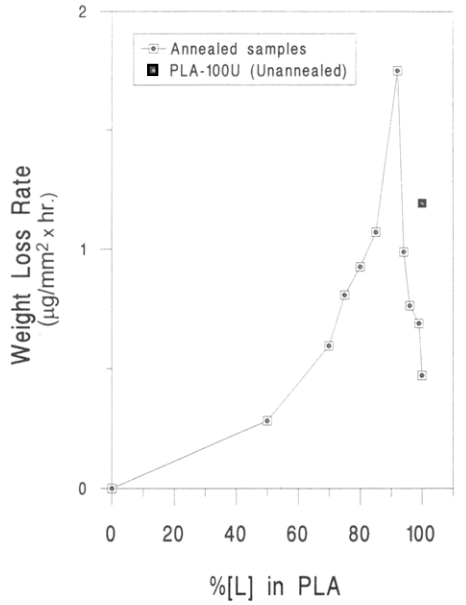

Figure 3. Film weight loss rates (($\mu g/mm^2$)/h) as a function of PLA stereochemical compositions. The solid square designates the PLA-100U sample. Rate values were obtained by measuring the slopes of the lines in Figure 2 and from additional normalized weight loss results for PLA-50, PLA-70, PLA-75, PLA-80, and PLA-85 film samples which were not displayed in graphical form.

unannealed) showed relatively much lower crystalline order as determined by the ΔH_f and percent crystallinity values (see Figure 1 and Table 2), the opportunity therefore arose to compare the enzymatic degradation rates of these two respective samples that have identical stereochemical structure but dramatically different crystalline morphology.

PLA/Enzyme Incubations. Film Weight Loss Studies. Figure 2 shows the normalized weight loss (in $\mu g/mm^2$) after exposure of PLA films with stereochemical compositions from 92 to 100%-L to proteinase K (see Experimental Section). It is interesting to note that the normalized weight loss values shown in Figure 2 for film percent weight loss values of less than ca. 60% appeared linear as a function of time. Therefore, slopes of the lines generated in Figure 2 were calculated by linear regression analysis (see ref 42 for correlation coefficient values) and are used herein as a measure of the rate of film weight loss. The rate values thus obtained as a function of the % L repeat unit content of the polymer are shown in Figure 3. When the film–enzyme exposures were continued beyond a 60% film weight loss, complete film disappearance was observed without any residual solid particulate matter, confirming that the PLA samples were completely degradable by proteinase K to water-soluble products.

Of primary importance to a discussion of the results in Figures 2 and 3 is that the PLA-0 (100% D) film showed no apparent weight loss. Thus, the (L)-lactide as opposed to (D)-lactide containing polymer chains are preferred substrates for proteinase K enzymatic degradation. In addition, control runs where the PLA samples were incubated over identical exposure conditions in the absence of enzyme showed no measurable film weight loss so that the observed film weight loss in the presence of enzyme is due to enzyme-catalyzed processes. Observation of Figure 3 shows that PLA-92 is clearly the most rapidly degraded by proteinase K for stereochemistries between 92 and 100% L. The relative order of PLA enzymatic degradation as viewed in Figure 3 is as follows: PLA-92 > PLA-94 > PLA-96 > PLA-99 > PLA-100. Based on structural considerations alone, it would be expected that the rate of proteinase K degradation of PLA would decrease with decreased % L repeat unit composition, which is precisely opposite to the result obtained. Therefore, it can then be concluded that the dominant factor determining the relative rate of PLA degradation within this narrow range of PLA stereochemistries is the degree of film crystalline order. It is important to note that a dramatic increase in the film weight loss rate value from 0.99 to 1.75 ($\mu g/mm^2$)/h was observed for PLA-94 and PLA-92, respectively. Thus, for only a 2% change in PLA stereochemical composition, dramatic changes in enzyme degradation rate were achieved. This large enhancement in degradation rate is attributed to a critical degree of crystalline order disruption that must have been achieved at the 92% L stereochemistry. The decreased crystalline order for PLA-92 relative to PLA-94 is reflected not only in the respective percent crystallinity values determined by WAXS (see Table 2) but also by the decreased degree of crystalline perfection indicated by the respective T_m and ΔH_f values for these two samples (see Figure 1). A similar dramatic increase in rate was observed in previous work by our laboratory on poly(β-hydroxybutyrate) (PHB) stereocopolymers.[31] In that work, measurements were carried out specifically on the initial surface degradation rate. The unexpectedly large increase in the PHB initial surface degradation rate at a 77% *R* stereochemical composition also occurred where the degree of sample crystalline order was significantly disrupted. It is important to note that the M_n for PLA-92 is approximately equal to that of PLA-94 (M_n = 219 000 and 244 000, respectively) and is greater than that of PLA-100 (M_n = 198 000) so that the small differences between PLA stereocopolymer molecular weight values could not be used to explain the above-discussed rate differences.

An identical weight loss study was carried out on PLA stereocopolymer films containing 50, 70, 75, 80, and 85% L repeat units (weight loss curves not shown). In this study as above the normalized weight loss as a function of time was approximately linear to 60% film weight loss. Using linear regression analysis (see ref 42 for correlation coefficient values), slopes of the normalized weight loss as a function of time provided quantitation of the rate of film weight loss. From observation of the slope values shown in Figure 3, the maximum weight loss rate was

observed for the PLA-92 sample. Also, the rate of film weight loss continuously decreased for PLA stereocopolymers with % L contents from 92 to 50% (see Figure 3). Interestingly, a dramatic decrease in rate was observed for PLA-85 relative to PLA-92. Differences in molecular weight between PLA-85 and PLA-92 (M_n = 169 000 and 219 000, respectively) might, if anything, only enhance the rate differences between these two samples. Of importance in this discussion is the fact that the PLA-85 sample was amorphous. Decreased crystallinity and PLA L content for PLA-85 relative to PLA-92 represent variables that, when considered alone, are expected to increase and decrease the observed degradation rate, respectively. Therefore, it is apparent that the disruption of crystalline order observed for the PLA-92 sample is sufficient to allow the PLA L content to dominate the observed degradation rate. Furthermore, the disproportionally large decrease in PLA degradation rate associated with only a 7% decrease in L content for these two respective samples indicates that the dependence of enzyme-catalyzed film weight loss rates on polymer stereochemistry may, for specific enzyme/polymer systems, be nonlinear.

The observation of decreased PLA film weight loss rate for PLA stereocopolymers with % L contents from 85 to 50% (see Figure 3) was expected since, in this range of PLA stereochemical compositions, the resulting films were all amorphous. Effects of decreased sample molecular weight for PLA-70 and PLA-75 relative to PLA-80 and PLA-85 (see Table 1) did not cause any apparent complications in the general trends observed in this % L compositional range. This point is clarified by noting that PLA-70 and PLA-75 are of lower molecular weight than PLA-80 but still show decreased PLA film weight loss rates relative to PLA-80. Thus, in the absence of crystalline morphology effects, the rate of film weight loss decreases with decreased polymer % L repeat unit content due to a preference by proteinase K for the (L)-PLA enantiomeric form.

Investigation of the film weight loss rates for PLA-100 and PLA-100U provided an opportunity to study effects of film crystallinity as an independent variable. These two samples have identical repeat unit stereochemistries and nearly identical M_n values (see Table 1) while having differences in percent crystallinity as measured by WAXS of 57 and 27%, respectively (see Table 2). The increase in percent crystallinity by approximately a factor of 2 resulted in a large decrease in the film weight loss rate from 1.19 to 0.47 (μg/mm^2)/h for PLA-100U relative to PLA-100. The enhanced rate at lower degrees of crystallinity is, of course, expected based on previous studies on poly(ϵ-caprolactone) by Huang and co-workers[43–45] and on natural-origin PHB by Nishida and Tokiwa.[46] It is interesting to note that PLA-100U has a slower film weight loss rate relative to PLA-92 even though percent crystallinity values (by X-ray diffraction) of these two samples are similar (27 and 32%, respectively). This result is not surprising since the PLA-100U sample has higher T_m and ΔH_f values relative to PLA-92 (182 and 148 °C and 2.7 and 1.4 cal/g, respectively), suggesting that PLA-100U has a higher ordered crystalline phase (less crystalline defects). The formation of a relatively more thermodynamically stable crystalline phase is expected to lead to correspondingly slower rates of film weight loss. Also, it is important to recognize that the magnitude of the rate enhancement as a function of changes in crystalline order is not readily predictable and should be dependent on the specific enzyme/polymer system under study.

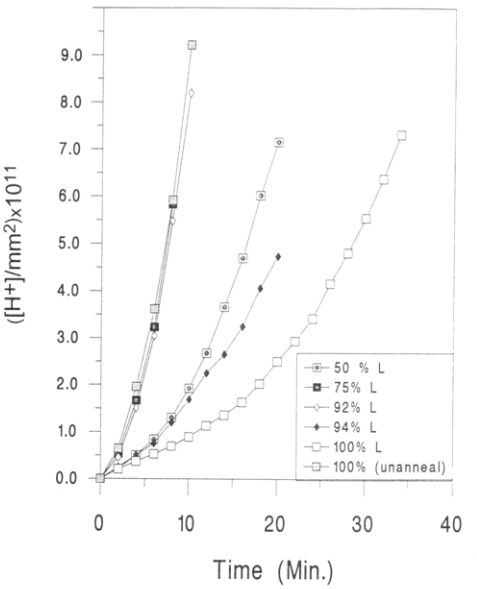

Figure 4. Initial surface degradation ([H$^+$]/mm^2) as a function of time for PLA stereoisomer/proteinase K incubation studies. The curves were generated from data points collected every 2 min.

Initial Surface Degradation Rate Measurements. Analyses were performed to determine the effect of PLA stereochemical composition on the initial surface degradation rate. These measurements were carried out by exposure of PLA films to proteinase K and monitoring of changes in pH (see Experimental Section).[31] Control experiments carried out in the absence of enzyme addition showed no significant change in solution pH over the time interval studied. Therefore, monitoring of pH change provided us with a very sensitive method to measure, over short time intervals (minutes), the production of H$^+$ ions, which directly corresponds to the number of events of chain cleavage.[31] The results of this work are shown in Figure 4, where the H$^+$ ions produced as a function of time were divided by the initial film surface area (mm^2) to normalize for effects due to the film dimensions.[31] This treatment of the data is important since the substrate concentration can best be defined in this experiment by the available film surface area. The [H$^+$]/mm^2 values reported in Figure 4 are the average of three independent measurements (see Experimental Section). Observation of Figure 4 shows that all of the generated curves have an initial relatively slower rate of [H$^+$]/mm^2 ion generation followed by a relatively more rapid secondary rate of [H$^+$]/mm^2 ion liberation. The initial time period required before the establishment of a relatively more rapid degradation process is normally associated with a lag phase. The observed lag phases may be attributable to the respective time periods required for maximum protein absorption to the substrate film surface. The rates of [H$^+$]/mm^2 generation which followed the lag phases were analyzed by linear regression analyses, and the calculated slope values are described herein as the initial rates of surface degradation. A plot of the initial rates of surface degradation as a function of the PLA stereocopolymer composition is displayed in Figure 5. The confidence limits of the results shown in Figure 5 shown as error bars were calculated from three independent measurements of [H$^+$]/mm^2 as a function of time. Initial inspection of the results in Figure 5 show a dramatic increase in rate for PLA-92 relative to PLA-94 and a maximum rate value for PLA-92. This result is in excellent agreement with that observed in Figure 3, where the rate of film weight loss was measured. Further inspection of Figure 5 shows no significant

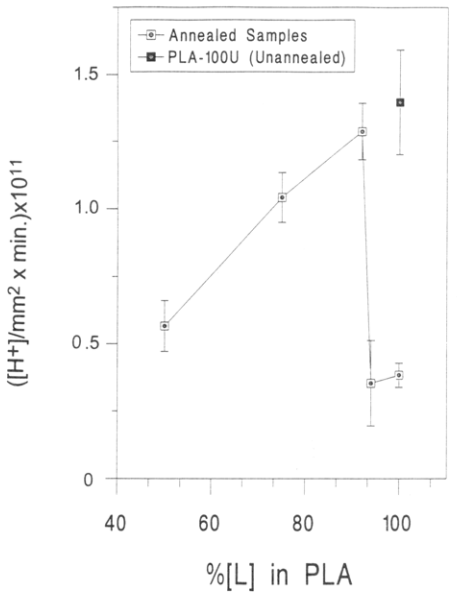

Figure 5. Enzyme degradation rates (([H^+]/mm^2)/min) as a function of PLA stereochemical composition for initial surface degradation studies. The solid square designates the PLA-100U sample. The rate values were obtained by measuring the slopes of the linear regions of the curves (post-lag period) displayed in Figure 4.

difference in the measured initial surface rate values for PLA-100 and PLA-94. In contrast, PLA-94 showed a much more rapid film weight loss rate than PLA-100 (see Figure 3). It should be noted that important differences between the PLA-100 and PLA-94 samples were observed for the respective lag phases, where the former relative to the latter sample showed a significantly extended lag time period (see Figure 4). It may be speculated that increases in the lag phase corresponds to a lower binding affinity of the enzyme to a PLA substrate. Of additional interest is the relatively slow decrease in initial surface degradation rates with decreased % L contents from 92 to 50% whereas the rate of film weight loss decreased more rapidly as a consequence of higher PLA D repeat unit content (see Figures 3 and 5). This may be explained by the presence of sufficient concentrations of L repeat units at the surface to sustain high rates of initial surface degradation. One may speculate that if prolonged initial surface degradation measurements on samples such as PLA-50 had been carried out, decreased surface degradation rates may be observed due to a utilization of L-rich repeat unit sequences, thereby leaving a surface enriched in D repeat units. It is interesting to note that unexpectedly rapid rates of initial surface degradation followed by a decrease in the surface degradation rate for prolonged incubation times were reported for PHB stereocopolymers that contained high contents of the less preferred repeat unit stereoisomer (33 and 50% (*S*)-β-hydroxybutyrate).[31]

The rates of initial surface degradation for PLA-100U (unannealed) and PLA-100 were compared. As discussed above, comparisons of the degradation rates of these two samples are of particular importance since they allow study of crystalline morphology effects in the absence of changes in polymer structure. Interestingly, the PLA-100U sample of relatively lower percent crystallinity (see Table 2) showed a dramatic increase in the rate and decrease in the lag time period relative to PLA-100. This result is consistent with the rate enhancements reported above by film weight loss measurements for PLA-100U relative to PLA-100.

Table 3. Molecular Weight and Thermal Measurements on Recovered PLA-100 Film Samples after Enzyme Exposures

time of degrad (h)	% wt loss	M_w[a]	M_w/M_n[a]	heat of fusion (cal/g)[d]
0	0	314 000 ± 7500[b]	1.69	9.1 ± 0.3[e]
12	17.5	313 000	2.08	9.7 ± 0.1[e]
24	25.5	308 000	1.99	10.1 ± 0.2[f]
36	44.6	ND[c]	ND	9.9 ± 0.02[f]
48	65.5	ND	ND	10.9

[a] Determined by GPC (see Experimental Section). [b] Standard deviation calculation based on three replicate samples. [c] Not determined. [d] Determined by DSC (see Experimental Section). [e] Standard deviation calculation based on three replicate samples. [f] Standard deviation calculation based on two replicate samples.

In this study, it can be concluded that the rates of initial surface degradation and film weight loss measurements are, qualitatively, in agreement. Of importance when utilizing initial surface degradation results to predict film weight loss measurements is that the former shows a relatively high tolerance to the stereoisomer which is less preferred by the degrading enzyme. Also, the lag phase time period provides information in addition to that from the initial surface degradation rate values that can prove useful as well.

To better interpret the above initial surface degradation results and the corresponding lag periods, detailed information on the rate and extent of enzyme binding to the film surface as a function of PLA stereochemistry is needed. In addition, correlations between the degree of order in the film bulk and at the film surface would be of great interest. Efforts to obtain such information on this and related polymer substrate–enzyme systems are in progress in our laboratory.

Effects of Enzyme Exposures on Residual PLA Films. Residual PLA-100 films recovered after enzyme exposure times of variable duration were analyzed for changes in molecular weight and crystallinity by GPC and DSC (see Experimental Section), respectively. The results of this study showed that after a loss in film weight of 25.5% the residual PLA sample shows little molecular weight change (see Table 3). This is typical of an enzyme-catalyzed film degradation process which occurs at the film surface.[47] In such a process, the enzyme cannot diffuse into the film bulk and the mass fraction of polymer chains which are enzyme accessible at the film surface is small relative to the mass fraction of polymer chains which remain nondegraded in the film bulk material. Of course, to achieve such high degrees of film weight loss by chemical hydrolysis, a large decrease in the molecular weight of the PLA sample would be required.[48] Table 3 also shows that as the enzyme degradation process proceeds from 0 to 65.5% film weight loss, an increase in the ΔH_f from 9.1 to 10.9 cal/g was measured. This is consistent with other work on enzyme-catalyzed degradation of semicrystalline polymers which showed a preferential degradation of the sample amorphous domains.[8,27,28]

Summary of Results

The proteinase K catalyzed PLA film weight loss and initial surface degradation rates are greatly affected by the PLA stereochemical composition. Since proteinase K preferentially degrades (L)-PLA as opposed to (D)-PLA, it is expected considering structure as an independent variable that decreased L repeat unit content in PLA will lead to slower proteinase K catalyzed degradation rates. However, decrease in the PLA % L content over the narrow compositional range from 100 to 92% leads to a large decrease in crystalline order while PLA-85 was amorphous.

It was found that both the initial surface and film weight loss rates had optimal values for PLA-92 so that effects of crystalline morphology dominated the observed rate values for stereochemical compositions from PLA-100 to PLA-92. A rapid decline in the film weight loss rate as the % L content is decreased from 92 to 85% in combination with a dramatic increase in the film weight loss rate as the % L content decreased from 94 to 92% indicates that a critical degree of disruption in the crystalline order is associated with PLA having approximately 92% L repeat units. This explanation would account for the fact that even though the percent crystallinity decreased from 32 to 0% for PLA-85 relative to PLA-92, PLA-85 still showed significantly decreased film weight loss rates. Thus, once a critical degree of disruption of the crystalline phase was reached, the structural effects caused by changes in PLA stereochemical composition dominate the observed degradation rate. Initial surface degradation rates were unexpectedly high for PLA-75 and PLA-50 relative to PLA-92, which was discussed in regards to the sufficient availability of chain segments at the film surface which have high % L contents. In contrast, a rapid decline in film weight loss rate values was found for PLA stereochemical compositions from 92 to 50% L. However, qualitatively, the rates of initial surface degradation and film weight loss measurements are in agreement. Residual PLA-100 from enzyme incubations where film weight loss exceeded 25% showed little change in molecular weight and increased ΔH_f values as was expected for an enzyme-catalyzed film degradation process that takes place at the film surface and occurs preferentially at amorphous domains.

Acknowledgment. The authors are grateful for the financial support received from the donors of the Petroleum Research Fund, administered by the American Chemical Society (PRF, No. 24695-AC7P), and the National Science Foundation, Division of Materials Research, under a Presidential Young Investigator Award (R.G., Grant DMR-9057233).

References and Notes

(1) Heller, J. *Biomaterials* **1980**, *1*, 51.
(2) Marcotte, N. Delayed Release of Water-Soluble Macromolecules from Polylactide Pellets, Master's Thesis, Department of Chemical Engineering, Queen's University, 1988.
(3) Jakanicz, T. M.; Nash, H. A.; Wise, D. L.; Gregory, J. B. *Contraception* **1973**, *8*, 227.
(4) Pennings, J. P.; Dijkstra, H.; Pennings, A. J. *Polymer* **1993**, *34* (5), 942.
(5) Vainionpaa, S.; Rokkanen, P.; Tormala, P. *Prog. Polym. Sci.* **1989**, *14*, 679.
(6) Pennings, J. P.; Dijkstra, H.; Pennings, A. J. *Polymer* **1993**, *34* (5), 942.
(7) 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.
(8) Leenslag, J. W.; Pennings, A. J.; Bos, R. M.; Rozema, F. R.; Boering, G. *Biomaterials* **1987**, *8*, 311.
(9) 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.
(10) 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.
(11) Gu, J.-D.; Gada, M.; Kharas, G.; Eberiel, D.; McCarthy, S. P.; Gross, R. A. *Polym. Mater. Sci. Eng.* **1992**, *67*, 351.
(12) Kim, S. H.; Han, Y.-K.; Kim, Y. H.; Hong, S. I. *Makromol. Chem.* **1991**, *193*, 1623.
(13) Kricheldorf, H. R.; Serra, A. *Polym. Bull.* **1985**, *14*, 497.
(14) Kricheldorf, H. R.; Berl, M.; Scharnagl, N. *Macromolecules* **1988**, *21*, 286.
(15) Nijenhuis, A. J.; Grijpma, D. W.; Pennings, A. J. *Macromolecules* **1992**, *25*, 6419.
(16) Zhang, X.; Goosen, M.; Wyss, U. P.; Pichora, D. *J. Macromol. Sci., Rev. Macromol. Chem. Phys.* **1993**, *C33* (1), 81.
(17) Lavallee, C. *Proc. Int. Symp. Adv. Polym. Synth., Aug 26–31, 1984*, **1985**, 441–461.
(18) Dubois, Ph.; Jacobs, C.; Jerome, R.; Teyssie, Ph. *Macromolecules* **1991**, *24*, 2266.
(19) Trofimoff, L.; Aida, T.; Inoe, S. *Chem. Lett.* **1987**, 994.
(20) Kricheldorf, H. R.; Boettcher, C. *Makromol. Chem.* **1993**, *194*, 463.
(21) Grijpma, D. W.; Jozaisse, C.; Pennings, A. J. *Makromol. Chem., Rapid Commun.* **1993**, *14*, 155.
(22) Kricheldorf, H. R.; Kreiser-Saunders, I. *Makromol. Chem.* **1990**, *191*, 1057.
(23) Bero, M.; Kasperczyk, J.; Adamus, G. *Makromol. Chem.* **1993**, *194*, 907.
(24) Grijpma, D. W.; Zondervan, G. J.; Pennings, A. J. *Polym. Bull.* **1991**, *327*.
(25) Miller, R. A.; Brady, J. M.; Cutright, D. E. *J. Biomed. Mater. Res.* **1977**, *11*, 711.
(26) Vert, M.; Chabot, F.; Leyray, J.; Christel, P. *Makromol. Chem. Suppl.* **1981**, *5*, 30.
(27) Fukuzaki, H.; Yoshida, M.; Asano, M.; Kumakura, M. *Eur. Polym. J.* **1989**, *25* (10), 1019.
(28) Chu, C. C. *J. Appl. Polym. Sci.* **1981**, *26*, 1727.
(29) Williams, D. F. *Eng. Med.* **1981**, *10* (1), 5.
(30) Makino, K.; Arakawa, M.; Kondo, T. *Chem. Pharm. Bull.* **1985**, *33* (3), 1195.
(31) Kemnitzer, J. E.; McCarthy, S. P.; Gross, R. A. *Macromolecules* **1992**, *25*, 5927.
(32) Ebeling, W.; Hennrich, N.; Klockow, M.; Metz, H.; Orth, H. D.; Lang, H. *Eur. J. Biochem.* **1974**, *47*, 91.
(33) By measuring the initial surface degradation rates of a PLA-94 film at the pH intervals from 9.0 to 8.5, 8.5 to 8.0, and 8.0 to 7.5, it was shown that the enzyme activity on the substrate was virtually identical within the pH range of 9.0–8.0 (Reeve, M. S.; Gross, R. A., unpublished results, 1993).
(34) Bloembergen, S.; Holden, D. A.; Hamer, G. K.; Bluhm, T. L.; Marchessault, R. H. *Macromolecules* **1986**, *19*, 2865.
(35) Cohn, D.; Younes, H. *J. Biomed. Mater. Res.* **1988**, *22*, 993.
(36) 12.8 units/mg of solid, 13.2 units/mg of protein (Biuret). One unit will hydrolyze casein to produce color equivalent to 1.0 μmol (181 μg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin–Ciocalteu reagent).
(37) Kricheldorf, H. R.; Boettcher, C.; Tonnes, K. *Polymer* **1992**, *33* (13), 2817.
(38) Kawai, F. *CRC Crit. Rev. Biotechnol.* **1987**, *6* (3), 273.
(39) Tanio, T.; Fukui, T.; Shirakura, Y.; Saito, T.; Tomita, K.; Kaiho, T.; Masamune, S. *Eur. J. Biochem.* **1982**, *124*, 71.
(40) Personal communication by Robert Vetrecin of Ethicon Division of Johnson and Johnson, Aug 11, 1993.
(41) Li, S.; Garreau, H.; Vert, M. *J. Mater. Sci., Mater. Med.* **1990**, *1* (4), 198.
(42) The correlation coefficients for the rate data presented in Figure 3 are as follows: PLA-0 (0.999); PLA-50 (0.998); PLA-70 (0.980); PLA-75 (0.999); PLA-80 (0.993); PLA-85 (0.999); PLA-92 (0.994); PLA-94 (0.999); PLA-96 (0.999); PLA-98 (0.998); PLA-99 (0.999); PLA-100 (0.994); PLA-100U (0.995).
(43) Cook, W. J.; Cameron, J. A.; Bell, J. P.; Huang, S. J. *J. Polym. Sci., Polym. Lett. Ed.* **1981**, *19*, 159.
(44) Benedict, C. V.; Cook, W. J.; Jarret, P.; Cameron, J. A.; Huang, S. J. *J. Appl. Polym. Sci.* **1983**, *28*, 327.
(45) Jarret, P.; Benedict, C. V.; Bell, J. P.; Cameron, J. A.; Huang, S. J. In Polymers as Biomaterials; Shalaby, S. W., Hoffman, A. S., Ratner, B. D., Horbett, T. A., Eds.; Plenum Press: New York, 1984; pp 181–192.
(46) Nishida, H.; Tokiwa, Y. *J. Environ. Polym. Degrad.* **1993**, *1* (1), 65.
(47) St. Pierre, T.; Chiellini, E. *J. Bioact. Compat. Polym.* **1987**, *2*, 4.
(48) Pitt, C. G.; Gratzl, M. M.; Kimmel, G. L.; Surles, J.; Schindler, A. *Biomaterials* **1981**, *2*, 215.