Copolymerization and Degradation of Poly(lactic acid-*co*-lysine)

Denise A. Barrera,† Eric Zylstra,† Peter T. Lansbury,† and Robert Langer*,‡

Departments of Chemistry and of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT: Poly(lactic acid-*co*-lysine) has been synthesized and the side-chain amino groups of the lysine residues have been modified with an RGD (arginine-glycine-aspartic acid) cell adhesion promoting peptide. In order for this modified copolymer to be useful as a matrix material for tissue engineering, it is critical to understand how the polymerization conditions affect the copolymer composition, molecular weight, crystallinity, and degradability. When 3-[N_ϵ-(carbonylbenzoxy)-L-lysyl]-6-L-methyl-2,5-morpholinedione was copolymerized with L,L-lactide at 136 °C for 48 h, increasing the concentration of the morpholinedione derivative increased the concentration of protected lysine residues in the resulting copolymers. Up to 10.6% of the protected lysine monomer could be incorporated into the copolymer structure. However, under these conditions low molecular weights (15 000) and yields (20%) were observed. The polymerization time and temperature were varied, and it was found that lower polymerization temperatures and longer times gave higher molecular weights, up to 94 500 with 75% yield. When exposed to pH 7.1 phosphate-buffered saline at 37 °C, the copolymers degraded to half of their original molecular weight values in 5 weeks compared to 15 weeks for poly(L-lactic acid). The faster degradation rate was due mainly to the disruption of crystallinity by the lysine residues in the copolymers.

Introduction

One challenge in the area of biomedical materials is the development of substances that can favorably interact with mammalian cells either *in vitro* or *in vivo*. Such materials could be useful for applications ranging from the basic study of how cells interact with surfaces to applied areas such as *in vitro* mammalian cell culture or *in vivo* cell transplantation. For example, isolated cells and cell clusters from donor organs or living donors could be placed onto scaffolds *in vitro*, and then the resulting polymer-cell device could be implanted into patients in need of organ replacement. This emerging field of tissue engineering holds great promise for the millions of patients who suffer tissue loss or organ failure each year.[1]

One challenge in tissue engineering is the development of suitable matrix materials, for cell function and viability are greatly affected by the substrate.[2] Natural substances such as collagen and fibronectin contain information that supports cell adhesion and differentiated cell function,[3] and several research groups have modified synthetic nondegradable polymers with biologically active moieties.[4–15] However, degradability may be important so that implanted cells can eventually obtain a completely natural environment, thereby eliminating the possibility of long-term detrimental tissue response. Poly(lactic acid) (PLA) is a versatile, well-characterized material and one of the few degradable materials currently used clinically (e.g., sutures).[16–18] However, there are no sites available on this material to modify its surface with biologically active moieties. An increasing number of potentially degradable polyesters that have side chains with functional groups are being investigated, and a review of these systems is given by Veld.[19] However, we are not aware that any of these systems have been modified with ligands known to specifically regulate cell function.

In a previous paper,[20] we described the initial synthesis of poly(L-lactic acid-*co*-L-lysine) as well as the chemical attachment of the peptide sequence GRGDY to the lysine residue in the copolymer. This approach combines the advantages of both natural and synthetic materials. In order for this modified copolymer to be useful as a matrix material for tissue engineering, it is critical to understand how the polymerization conditions affect the copolymer composition, molecular weight, crystallinity, and degradability. In this paper, we discuss the relationship between the copolymerization conditions and the poly(lactic acid-*co*-lysine) properties including hydrolytic degradability.

Experimental Section

Materials. N^ϵ-(Carbonylbenzoxy)-L-lysine and D-alanine were purchased from Sigma. The plates for the TLC were purchased from Analtech and were hard layer silica gel HLF uniplates with an organic binder and a fluorescent indicator UV254. The column for the silica gel column chromatography was from Ace Glass, while the silica gel was silica gel 60, 70–230-mesh, purchased through VWR. L,L-Lactide was purchased from Polysciences. Stannous octoate was purchased from Aldrich and used without further purification. SurfraSil was purchased from Pierce and used as a 10% solution in dry toluene. Ninhydrin was purchased from Fluka. Sodium propionate, 2-methoxyethanol, propionic acid, 6-aminohexanoic acid, palladium chloride, triethylsilane, triethylamine, and 1,1′-carbonyldiimidazole were also purchased from Aldrich. The GRGDY peptide was synthesized by the Biopolymers Laboratory, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA. The lactic acid assay reagent and standard were purchased from Sigma. Sigma refers to this reagent as a lactate reagent.

Methods. Molecular weight data were collected on a Perkin-Elmer GPC system consisting of a Series 10 pump, an LKB 2140 rapid spectral detector at 259 nm, an LC-25 refractive index detector, and a PE 3600 data station. The eluent was chloroform, and the column was a mixed-bed Phenogel column with 5-μm particles from Phenomenex. The molecular weights were determined relative to narrow molecular weight (polydispersity index ≤1.05) polystyrene standards from Polysciences. Thermal transition data were collected with a Perkin-Elmer DSC-7. The sample size ranged from 2 to 8 mg, and indium was used for both the temperature

* To whom correspondence should be addressed.
† Department of Chemistry.
‡ Department of Chemical Engineering.
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and enthalpy calibrations. Each sample was subjected to a heat−cool−heat cycle from 0 to 200 °C with rates of +20, −20, and +10 °C/min, respectively. The values reported are from the second heating cycle, except for the monomers which were only heated once. Specific rotations were measured in dioxane at room temperature, 23 °C, on a PolyScience Corp. SR-6 polarimeter. IR spectra were recorded on a Perkin-Elmer 1420 ratio recording instrument. UV spectra were recorded on a Perkin-Elmer 553 fast scan UV/vis spectrophotometer. ^{13}C NMR were recorded on an instrument from Bruker operating at 50 MHz. ^1H NMR were recorded on two instruments from Bruker operating at 200 and 250 MHz, respectively. Abbreviations used include singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and unresolved multiplet (m). Mass spectra were recorded on a Finnigan 8200 mass spectrometer using electron impact ionization. Elemental analysis was performed by Galibraith Laboratories, Knoxville, TN.

Monomer Synthesis. D-α-Bromopropionyl Chloride (3). D-Alanine (50 g, 0.56 mol) was dissolved in a mixture of 580 mL of 48% aqueous HBr and 800 mL of water, and cracked ice was added to give a total volume of 3.4 L. $NaNO_2$ (104.3 g, 1.51 mol) was added in small portions with stirring, followed by 700 g of Na_2SO_4. When the stirred reaction had warmed to 15 °C, it was decanted from the solids and divided into three portions. Each portion was extracted four times with 125 mL of Et_2O. All the Et_2O was combined, dried over Na_2SO_4 and then $CaCl_2$, and then concentrated by evaporation. The entire procedure was repeated up to this point. The two batches were combined and distilled under vacuum using aspiration. The vacuum was not determined, but a forerun was collected initially from about 25−60 °C, while the product was collected from 100 to 104, 103 to 116, and 110 to 122 °C on three different occasions (lit.[21] 104−108 °C at 25 Torr). The yield was 60% (103.9 g, 0.68 mol). Next, 70 mL (114.5 g, 0.96 mol) of $SOCl_2$ was added to the distilled product. This reaction was heated to 60 °C for 8 h and then left at room temperature overnight. The reaction mixture was distilled under vacuum using aspiration. The vacuum strength was not determined, but the product was collected from 30 to 36, 30 to 56, and 31 to 56 °C on three different occasions. The yield was 74% (86.3 g, 0.50 mol). $[\alpha]^{23}_{589}$ +23.6 (0.04324 g/mL of dioxane) [lit. $[\alpha]^{15}_{578}$ −27.1 (neat) for the L compound from online data retrieval form the Beilstein database]: IR (neat, cm^{-1}) 1775 (CO); ^1H NMR ($CDCl_3$) δ 1.93 (d, J = 6.9 Hz, 3H, CH_3), 4.65 (q, J = 6.9 Hz, 1H, CH).

(*R*)-*N*²-(2-Bromo-1-oxopropyl)-*N*⁶-[(phenylmethoxy)carbonyl]-L-lysine (4). In a typical reaction, *N*⁶-[(phenylmethoxy)carbonyl]-L-lysine (96.4 g, 0.34 mol) was added with stirring to 2000 mL of chloroform which had been dried over molecular sieves. A slurry formed and was stirred for 20 min. D-α-Bromopropionyl chloride (**3**) (26.9 g, 0.16 mol) was added to 200 mL of chloroform, and this mixture was added over 5 min to the slurry of protected lysine. The total volume was brought to 3200 mL by the addition of more chloroform. The reaction was covered with a blanket of argon and allowed to react for 24 h. The reaction mixture never became a clear solution. The reaction was followed by TLC. When the reaction was determined to be complete, the products were purified. The solids still remaining at the end of the reaction were removed by filtration. It was necessary to thoroughly rinse these solids with additional chloroform in order to extract all of the product, which was soluble in the chloroform. After removing the solids, the solution was concentrated by evaporation and used in the next step without further purification. However, a sample was purified by silica gel chromatography to complete the chemical analysis: TLC R_f 0.26 (chloroform/methanol/acetic acid 95/5/3); IR ($CHCl_3$, cm^{-1}) 3500−2400 (carboxylic acid OH) 3440, 3390, 3310 (amide and carbamate NH), 3100−3000 (aromatic), 1740−1640 (several overlapped peaks, carboxylic acid, carbamate, and amide I); ^1H NMR ($CDCl_3$) δ 1.44 (m, 4H, 2 CH_2's), 1.74−1.85 (doublet over m, J = 6.9 Hz, 5H, CH_2, CH_3), 3.18 (m, 2H, CH_2 next to carbamate), 4.44 (q, J = 6.9 Hz, 1H, BrC**H**CH_3), 4.56 (m, 1H, HNC**H**CH_2), 5.1 (m, 3H, CH_2Ph and amide **NH**), 7.2−7.3 (m, 6H, aromatic protons and carbamate NH), 9.78 (bs, 1H, COO**H**); MS *m/z* (relative intensity) 334 ([M − HBr]⁺), 309 (1.15), 307 (1.16), 228 (25.6), 128 (33.4), 108 (28.0), 91 (100), 79 (23.4). Anal. Calcd for $C_{17}H_{23}BrN_2O_5$: C, 49.17; H, 5.58; Br, 19.24; O, 19.26. Found: C, 48.29; H, 5.77; Br, 17.28; O, 18.93.

(3*S*-*cis*)-Phenylmethyl Ester−[4-(6-Methyl-2,5-dioxo-3-morpholinyl)butyl]carbamic Acid or 3-[*N*⁶-(Carbonylbenzoxy)-L-lysyl]-6-L-methyl-2,5-morpholinedione (5). In a typical reaction, $NEtiPr_2$ (24.6 mL, 0.14 mol; 90% of maximum) was diluted with 50 mL of chloroform and added to the (*R*)-*N*²-(2-bromo-1-oxopropyl)-*N*⁶-[(phenylmethoxy)carbonyl]-L-lysine (**4**) (65.2 g, 0.16 mol; maximum if 100% yield from previous reaction) which was dissolved in chloroform from the previous step. The reaction was heated to reflux for 8 h and followed by TLC. When the reaction was complete, the solvent was removed by evaporation, and the sample was put under high vacuum. The sample was a waxy solid which would turn to a hard powder if put under vacuum long enough (1 week). This crude reaction product was purified by silica gel column chromatography. A column of silica gel was prepared. The eluent was chloroform/methanol/acetic acid in a ratio of 95/5/3, and the column dimensions were 59 × 7.5 cm. The reaction product was applied to the column (≤20 g in 30 mL of eluent). The product eluted from 2600 to 3100 mL. The chromatography was repeated four times. The solvent was removed by evaporation. After this step a viscous residue remained in the flask. A white precipitate was formed when petroleum ether was added to the residue with stirring. The resulting white powder was dried under vacuum. The sample was recrystallized from ethyl acetate. The yield for last two reactions combined was 31% (16.6 g, 0.05 mol): TLC R_f 0.46 (chloroform/methanol/acetic acid 93/5/2); melting endotherm onset (5 °C/min) 136.2 °C; $[\alpha]^{23}_{589}$ −87.0 (0.01120 g/mL of dioxane), actually a sample with a proton ratio of 0.95/0.05; IR (KBr, cm^{-1}) 3330, 3220 (amide and carbamate NH), 3100−3000 (aromatic), 1750 (CO carboxylic acid), 1685 (CO carbamate and amide I), 1530 (amide II type from carbamate); UV (1.418 g/L of $CHCl_3$; (absorbance) 243.0 (0.54), 252.9 (0.69), 258.7 (0.84), 263.5 (0.71), 268.5 (0.43); ^1H NMR (DMF-d_6) δ 1.43 (d, J = 6.8 Hz, 3H, CH_3), 1.51 (m, 4H, 2 CH_2's), 1.7−2.0 (m, 2H, CH_2), 3.12 (m, 2H, CH_2 next to carbamate), 4.25 (td, J = 3.2 and 6.5 Hz, 0.03H, HNC**H**CH_2), 4.44 (t, J = 5.4 Hz, 0.97H, HNC**H**CH_2), 5.06 (s, 2H, CH_2Ph), 5.16 (q, J = 6.8 Hz, 1H, OC**H**CH_3), 7.19 (t, J = 5.4 Hz, 1H, carbamate N**H**), 7.2−7.4 (m, 5H, aromatic protons), 8.38 (s, 0.97H, amide **NH**), 8.52 (s, 0.03H, amide **NH**); ^{13}C NMR (DMF-d_6) δ 16.1 (CH_3), 22.6 (CH_2), 41.0 (CH_2NHCOO), 53.8 (CHNH), 66.0 (CH_2Ph), 74.78 ($CHCH_3$), 74.82 ($CHCH_3$), 128.28, 128.32, and 129.0 (aromatic carbons), 138.4, 169.6, and 170.1 (carbonyl carbons); MS *m/z* (relative intensity) 334 (M^+), 227 (18.0), 127 (13.4), 108 (72.8), 107 (23.4), 91 (100), 79 (24.0), 56 (21.1). High-resolution MS. Calcd for $C_{17}H_{22}N_2O_5$: 334.1529. Found: 334.1527.

Polymerization. All glassware was heated at 130 °C overnight and cooled under vacuum before use. The polymerization flasks were siliconized before use with SurfraSil. The monomers were crystallized from dry ethyl acetate (over molecular sieves) in a drybox the day before the polymerization reaction was to be started and were allowed to dry under high vacuum overnight.

The monomers were added to the polymerization flasks in a drybox. Next, the stannous octoate initiator was added in a small volume of dry chloroform. The samples were put under high vacuum (<30 μmHg) for 1.5 h to eliminate the chloroform used to add the catalyst. The flask was flushed five times with argon over this 1.5 h period. During the last 30 min of this vacuum cycle, the samples were gently heated with heating tape until a small amount of monomer could be seen sublimating at the top of the flask. At this point the flask was sealed by simply turning the stopcock and put into a constant temperature oven. The polymerization was stopped by putting the samples into the freezer. Purification was performed by dissolving the unpurified samples in chloroform and dropping them into an excess of methanol. The sample size was generally 0.7−1.5 g. Chemical characterization results are given in Table 1.

Lactic Acid Assay. The reagent was prepared according the manufacturer's directions. The standard was diluted to yield concentrations of 0.2−0.02 mg/mL of lactate. A volume

Table 1. Chemical Characterization of Selected Copolymer Samples

technique	mol % **5**[a]	results
elemental anal.	3.8	found (calcd for 1.9% lysine): C, 50.77 (50.93); H, 5.72 (5.68); N, 1.04 (0.70); O, 40.75 (42.68)
UV (nm)	0.0	12.4 g/L of $CHCl_3$ (absorbance), 243.0 (0.78)
	10.6	6.4 g/L of $CHCl_3$ (absorbance), 243.0 (1.27), 252.9 (1.34), 258.7 (1.52), 263.5 (1.34), 268.4 (0.97)
IR (cm^{-1})	0.0	3500 (OH end groups), 2995, 2940 (CH), 1760 (CO ester), 1450 (symmetrical CH_3)
	3.0	3500 (OH end groups), 2995, 2940 (CH), 1760 (CO ester), 1450 (symmetrical CH_3) Plus: 3400 (amide and/or carbamate NH), shoulder 1680 (CO amide I), 1520 (amide II and amide II like stretch from carbamate)
^1H NMR ($CDCl_3$)	0.0	δ 1.58 (d, $J = 7.1$ Hz, 3H, CH_3), 5.16 (q, $J = 7.1$ Hz, 1H, CH)
	10.6	δ 1.58 (d, $J = 7.1$ Hz, 3H, CH_3), 5.16 (q, $J = 7.1$ Hz, 1H, CH) Plus: δ 1.6–2.1 (br m, 3 CH_2's and CH_3), 3.17 (br m, CH_2 next to carbamate), 4.60 (br m, $HNCHCH_2$), 5.08 (s, CH_2Ph), 7.34 (s, aromatic protons)

[a] Determined by ^1H NMR. Incorporation of 10 mol % **5** actually yields only 5 mol % lysine since each molecule of **5** contains one lysine residue and one lactic acid residue.

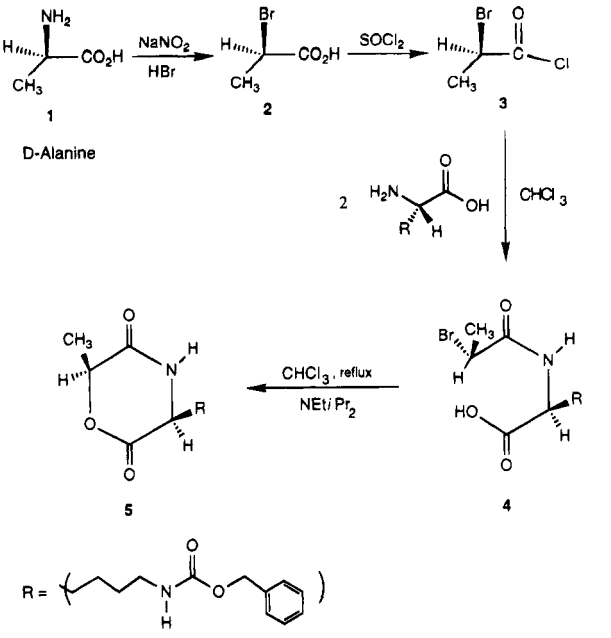
of 20 μL of either the standards or the samples was added to a disposable cuvette, and then 1 mL of the reagent was added. The solution was agitated gently and allowed to incubate for 5 min at room temperature. The absorbance was then measured against a reagent blank at 540 nm.

Solvent Casting. Glass beakers (10 mL, diameter 2.2 cm) were washed with chloroform and blown dry with a high-velocity nitrogen stream. Dust or other debris was also removed by this process. The copolymer (100 mg) was carefully weighed directly into the clean containers, and then 2 mL of chloroform was added. Up to 12 of the small beakers were put onto a flat glass surface, in this case a 15-cm-diameter Pyrex culture dish. These small beakers were then covered with a 12.5-cm-diameter, 6.5-cm-tall crystallizing dish. Vacuum grease was applied at the junction of the two glass pieces to control the evaporation. This method of controlling the evaporation rate was not very reproducible. The best films were obtained with an evaporation time of 48 h. After the films were dry, they were removed from the beakers and put under high vacuum to remove any residual solvent. If the films were cast on Teflon, they could be easily removed, but those cast on glass could not be removed without damaging the films. Consequently, the films in the glass beakers were covered with water for 4 h. After this treatment, the films could be removed with minimal damage, although it was still difficult occasionally. The wet films were allowed to air dry before being put under high vacuum.

Polymer Degradation. The polymer disks were produced by solvent casting as described above. The amount of polymer powder used for each disk was 102 ± 0.5 mg. However, after the processing was complete, the disks weighed between 110 and 115 mg. Residual solvent may account for this increase. Also, the first disk removed from the degradation buffer for all three types of samples weighed about 10 mg less even though no lactic acid was detected in the buffer. This could be due to the loss of residual solvent. However, since the source of the increased weight was not confirmed, the higher values were used as the initial weights.

Each polymer disk was accurately weighed and decontaminated by exposure to UV radiation for 15 min/side. The sterile disks were transferred to sterile 30-mL polyethylene vials with

Scheme 1. Synthesis of 5, the Cyclic Dimer of L-Lactic Acid and Protected L-Lysine



flip tops. The disks were covered with 10 mL of sterile pH 7.1 phosphate-buffered saline (PBS). The flip tops were closed, and the vials were transferred to a 37 ± 1 °C incubator and rotated at 120 rpm. Every 7 days, the buffer was removed and replaced with fresh buffer under sterile conditions. The buffer samples collected in this manner were stored at 4 °C and were analyzed for lactic acid content within 2 weeks. The pH of the buffers did not change.

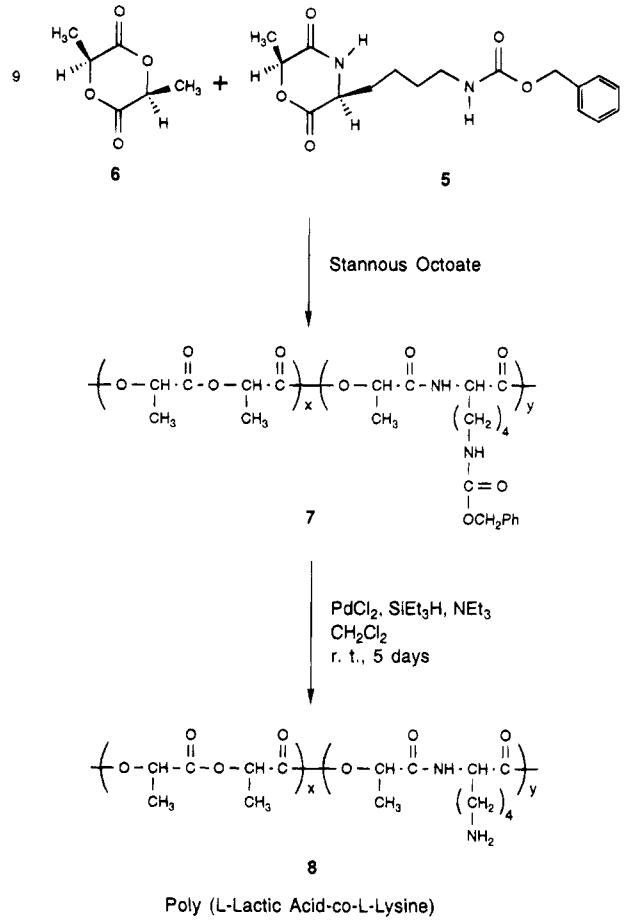
When the samples were removed from the degradation buffer, they were rinsed for 15 min in distilled water, allowed to air dry, and then vacuum-dried for at least 48 h before being subjected to the various analysis techniques.

Results and Discussion

In order to produce a copolymer of lactic acid and lysine, it was first necesary to synthesize a monomer that could polymerize by the same ring-opening mechanism used to synthesize PLA. An outline of the synthesis of 3-[N^{ϵ}-(carbonylbenzoxy)-L-lysyl]-6-L-methyl-2,5-morpholinedione (**5**) is provided in Scheme 1 as previously reported.[20] During the final ring-closure step, a minor amount of epimerization occurred; however, the diastereomeric purity was in excess of 95%. An attempt was also made to synthesize 3-[N^{ϵ}-(carbonylbenzoxy)-L-lysyl]-6-D-methyl-2,5-morpholinedione by starting with L-alanine. However, due to increased steric hindrance from the methyl group, additional epimerization occurred during the ring-closing step, resulting in a diastereomeric purity of only 75%. Veld et al.[19] have also reported the synthesis of **5** as a random diastereomeric mixture. Since no easy separation of the two cyclic diastereomers was available, the following polymerization studies were conducted using the more diastereomerically pure monomer, **5**.

The polymerizability of **5** is expected to be low due to the quantity and size of the substituents.[22] However, since the RGD adhesion moieties are very active, the lysine residues in the copolymer do not need to be present in very large concentrations. For example, only 1 fmol/cm^2 of the RGD adhesion peptide is necessary to cause cells to adhere to a previously nonadherent surface.[4] This value corresponds to a copolymer content of only 0.000 072 mol % lysine, assuming a polymer density of 1 g/cm^3 and an access layer 10 Å. Because

Scheme 2. Synthesis of Poly(L-lactic acid-*co*-L-lysine)



many factors may decrease the availability of the active sites, the lysine content of the copolymer must exceed the low value cited above. However, homopolymerization of **5** might actually be undesirable because it would produce a copolymer with 50 mol % lysine and 50 mol % lactic acid. At this concentration of lysine, the desirable physical characteristics of poly(lactic acid) could be lost, and the degradability might be disrupted. The desired concentration of lysine is 1−10 mol %. In this range, the degradability and good physical strength characteristics should be retained.

The copolymerization of **5**, which contains a protected lysine residue, and L,L-lactide (**6**) is shown in Scheme 2. The deprotection of the lysine residue has been reported previously[20] and is only shown for completeness. Several sets of copolymerization experiments were conducted to obtain copolymers of **5** and **6**. The first set of experiments dealt with controlling the copolymer composition, which is an important factor in determining the properties of the copolymer. Proton NMR and several other techniques were used to confirm that the protected lysine residues were incorporated into the copolymer. These results are summarized in Table 1. When the protected lysine concentration was high, the protected lysine residues were observed throughout the entire molecular weight distribution by UV. Table 2 shows that increasing the initial concentration of **5** in the reaction mixture increased the amount of lysine in the copolymer. However, only 40−50% of the available lysine residues were actually incorporated into the polymer structure, which supports the hypothesis that **5** is less polymerizable than L,L-lactide. In comparison, Veld et al.[19] obtained similar molecular weight values and found that 80−90% of the lysine residues were incorporated into a copolymer when their diastereomeric mixture of **5** was copolymerized with D,L-lactide.

Table 2. Effect of the Concentration of 5 on Polymerizations Conducted at 136 °C for 48 h Using a Catalyst to Monomer Ratio of 1/1000[a]

mole % 5[b]		yield			T_g	T_m
reaction	copolymer	(%)	M_n	M_w	(°C)	(°C)
0.0	0.0	85	132 000	223 000	61.6	169.4
5.3	2.6	71	14 500	36 700	57.5	155.3[c]
10.5	4.4	57	8 400	23 000	55.8	152.8[c]
27.7	10.6	20	12 300	15 000	52.4	none
100.0		0				

[a] All the molecular weight data were obtained on protected copolymers. [b] Determined by ^{1}H NMR. Incorporated of 10 mol % **5** actually yields only 5 mol % lysine since each molecule of **5** contains one lysine residue and one lactic acid residue. [c] Indicates 2 or more melting endotherms.

There were two undesirable effects resulting from the increase in the concentration of **5** in the reaction mixture. Both the molecular weight and the overall yield of copolymer decreased when the amount of **5** in the reaction was increased as shown in Table 2. During a polymerization reaction, the molecular weight can be lowered by both competing depolymerization reactions and other nonspecific chain scission reactions involving contaminants in the reaction mixture such as water. These nonspecific degradation reactions are expected to continue at the same rate regardless of the polymerization rate and thus will have a greater effect on the molecular weights of the slower polymerization. It is possible that higher concentrations of **5** decrease the polymerization rate, thereby decreasing the molecular weight. The lower polymerizability and perhaps also the slower kinetics of **5** were illustrated by an attempt at homopolymerization, which failed to yield any methanol-insoluble product at all. One explanation for the reduction in observed yield is that the methanol precipitation used for purification removes low molecular weight oligomers, with a cutoff around 10 000. Thus, the same mechanisms that cause the molecular weight reductions also tend to lower the observed yield.

The glass transition temperature, T_g, and the crystallinity of a polymer can greatly affect its end use properties. Table 2 shows that both the T_g and the melting temperature, T_m, of the samples decreased as the lysine monomer content in the reaction mixture was increased. The presence of the protected lysine residue at relatively low percentages disrupts the crystalline region slightly, causing the decrease in T_m and the appearance of two melting endotherms as indicated. This additional peak is quite small and appears about 10 °C lower than the dominant peak. As the lysine content in the copolymer increases, the crystalline region is eventually disrupted entirely, producing a completely amorphous polymer. Therefore, it may be possible to change the degradation rate of the copolymer structure by controlling the crystallinity. This approach is used to control the degradation rate of lactic acid/glycolic acid copolymers. However, amorphous polymers often have lower physical strength characteristics.

The molecular weights of all the copolymers in Table 2 are low in comparison to that of the PLA homopolymer. In order to ensure good processibility, it was expected that the molecular weights of the copolymers would have to be higher. Therefore, a set of polymerizations was completed where the monomer ratio was held constant at 90 mol % **6** to 10 mol % **5**, and the temperature was varied. Several time points were

Table 3. Effect of Time and Temperature on Polymerizations Using a 6 to 5 Mole Ratio of 90/10 and a Catalyst of Monomer Ratio of 1/1000[a]

temp (°C)	time (h)	M_w	yield (%)	mol % **5** in copolymer[b]
220	0.17	16 300	58	4.0
	0.33	21 100	56	3.8
	0.50	10 800	53	3.8
	1.00	9 700	43	5.0
	3.25	6 900	25	4.0
180	0.25	16 600	57	3.4
	0.50	15 100	46	4.8
	1.00	13 500	56	5.6
	2.00	12 000	55	4.4
	6.00	8 100	35	4.4
160	0.50	7 700	37	4.2
	2.00	13 700	61	5.2
	4.00	20 000	60	5.8
	8.00	15 400	49	5.4
	24.00	11 400	42	4.4
140	1.00	20 300	67	4.0
	4.00	23 600	60	6.4
	8.00	26 000	60	5.0
	24.00	12 600	60	5.0
	49.00	12 900	60	4.8
120	4.00	26 800	65	3.2
	8.00	33 400	68	3.2
	24.00	62 400	72	4.4
	73.00	43 000	72	5.0
	120.00	38 700	76	3.8
100	8.00	80 800	67	4.4
	24.00	84 100	76	3.4
	72.00	64 800	42	2.4
	168.00	63 700	79	3.8
	336.00	91 700	N/A	3.6
90	72.00	94 500	75	2.4
	474.00	87 000	78	3.8

[a] All the molecular weight data were obtained on protected copolymers. [b] Determined by proton NMR.

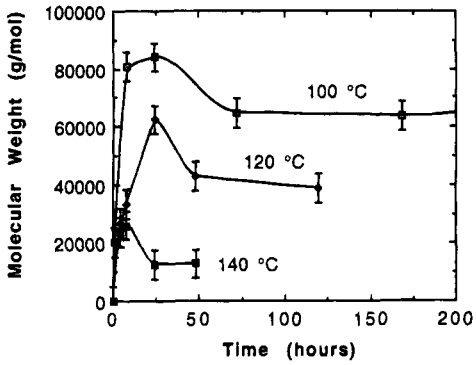


Figure 1. Time course of molecular weight (M_w). All the molecular weight data were obtained on protected copolymers.

taken for each temperature. The complete set of data from these experiments is shown in Table 3, while the results are summarized in Figures 1 and 2.

The results from this study are very similar to those observed for homopolymerizations of L,L-lactide using stannous octoate as the initiator.[23-25] Two major trends are observed. At each temperature, there is a maximum molecular weight as shown in Figure 1. This maximum is a result of the competition between the depolymerization and other degradation reactions versus the propagation reaction. As the overall monomer concentration decreases, the propagation reaction slows down. Eventually, the rate of degradation and depolymerization reactions taken together is faster than the propagation rate, and the molecular weight begins to decrease.

The second major trend is illustrated in both Figures 1 and 2. Lower polymerization temperatures produce

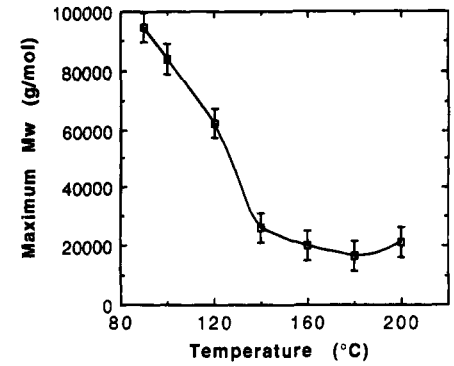


Figure 2. Maximum M_w for each polymerization temperature. All the molecular weight data were obtained on protected copolymers.

higher molecular weight polymers, but more time is required to reach the maximum molecular weight. There are three factors that may explain this increase in molecular weight with decreasing polymerization temperature.

The first factor is the possible existence of a ceiling temperature. The lower molecular weight values observed at the higher polymerization temperatures could be partially due to this phenomenon. Reported values for the ceiling temperature of L,L-lactide range from 275 to 640 °C.[24,26] The ceiling temperature of **5** is probably lower due to its lower polymerizability.

A second important factor is the relative rate of any other degradation reactions versus the propagation reaction. At higher temperatures all of the reactions will be accelerated, but the degradation reactions may be accelerated more than the propagation reactions. In this case, lower maximum molecular weights would be observed at earlier time points for the higher polymerization temperatures.

Finally, these polymers are partially crystalline, and the melting temperatures are generally around 150 °C. The presence of crystallinity could affect the polymerizations in two ways. First, the polymerizations conducted below the crystalline melting temperature should be more energetically favorable due to the crystallization of the polymer. Second, the degradation reactions that lower the molecular weight will probably be slower in the highly ordered crystalline regions. Both of these crystallinity effects would increase the observed molecular weights at polymerization temperatures below the crystalline melting temperature.

The final property of the copolymer to be characterized was the hydrolytic degradability. The copolymers for these studies were produced by polymerizing a 90/10 ratio of **6** to **5** for 24 h at 100 °C using a catalyst to monomer ratio of 1/1000. The *in vitro* degradation behavior for solvent-cast samples was determined at 37 °C in pH 7.1 PBS for three sample types: (1) poly(L-lactic acid) (PLA), (2) protected poly(L-lactic acid-*co*-L-lysine) (prot. PLA-*co*-Lys), and (3) the deprotected copolymer, poly(L-lactic acid-*co*-L-lysine) (PLA-*co*-Lys). The initial molecular weight (M_w) values were as follows: PLA, 115 300; prot. PLA-*co*-Lys, 74 100; and PLA-*co*-Lys, 39 400. Several variables were measured to characterize the degradation behvaior, including (1) visual inspection, (2) mass loss, (3) molecular weight reduction, (4) melting temperature, (5) heat of fusion, (6) lactic acid release, (7) copolymer composition by amino acid analysis, and (8) ^{1}H NMR.

The appearance of the PLA samples went from an opaque crystalline material to a white brittle material

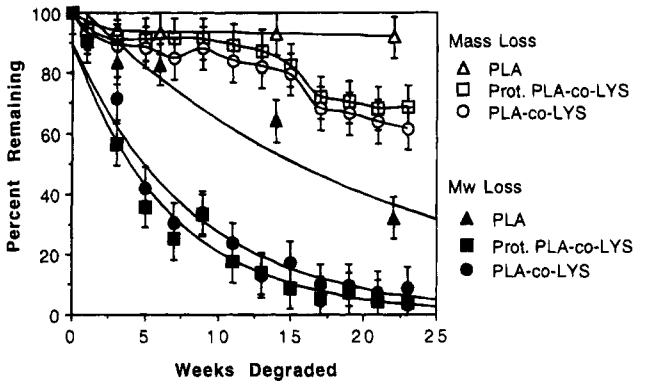


Figure 3. Normalized mass and molecular weight loss.

over the course of 22 weeks. The copolymers behaved in a similar manner except that the process was much faster. The Prot. PLA-*co*-Lys was quite brittle by 5 weeks, while the PLA-*co*-Lys was already brittle and broken at 3 weeks. The differences in the starting molecular weights are definitely responsible for these differences in physical strength reduction.

The mass and molecular weight losses for each of the three samples are shown in Figure 3. These results indicate that the degradation occurs in the bulk by random chain scission. Once the average molecular weight of the sample decreases to a certain level, chains become soluble and mass loss is observed.[27-31] The mass of the PLA sample remained steady through week 23 since the molecular weight had not gone below the critical value where the oligomers would be soluble in the PBS buffer. The mass and molecular weight losses for PLA are almost identical to values reported in the literature.[18]

The molecular weight decreases for the Prot. PLA-*co*-Lys and the PLA-*co*-Lys were very similar, as shown in Figure 3. This result indicates that the additional free amino groups do not change the degradation behavior significantly. However, the molecular weight decrease for the PLA was much slower than that of the copolymer samples. These different rates might be partially attributed to the difference in the starting molecular weight values, for samples with lower molecular weights have more end groups that could accelerate degradation rates. However, by week 10, the PLA sample had fallen to the initial molecular weight of the Prot. PLA-*co*-Lys. If the 10 week time point of the PLA molecular weight curve was moved to 100% remaining and 0 weeks, the results would still indicate that the PLA molecular weight decreases much slower than either the Prot. PLA-*co*-Lys or the PLA-*co*-Lys.

Another factor that could influence the observed difference in molecular weight decrease is the polymer crystallinity. The PLA has a higher crystalline melting temperature than either of the copolymer samples as shown in Figures 4 and 6. Higher melting temperatures within a polymer system imply that the crystallites are larger and have fewer defects. Due to the lysine residues, the crystallites in the copolymers are smaller and less perfect and therefore melt at a lower temperature. These smaller less perfect crystallites in the copolymers degrade more quickly than the larger defect-free crystallites of PLA. Therefore, the PLA-*co*-Lys molecular weight decreases more quickly mainly due to the disruption of the crystalline region by the lysine residues.

The complete set of melting temperature and heat of fusion data for the PLA-*co*-Lys are compared to the

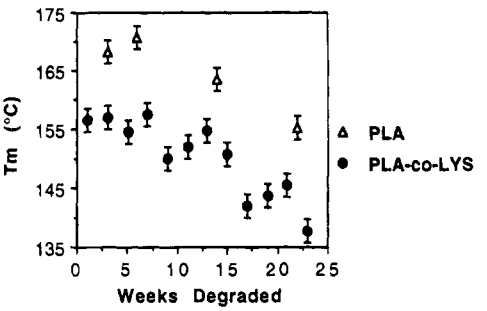


Figure 4. Melting temperatures for PLA-*co*-Lys and PLA.

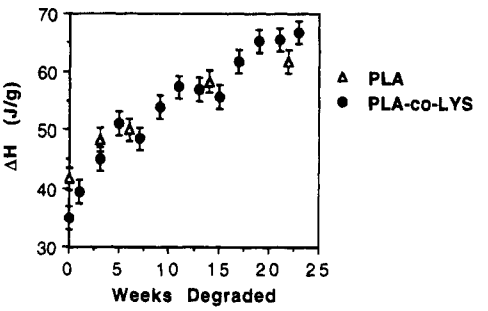


Figure 5. Heat of fusion for PLA-*co*-Lys and PLA.

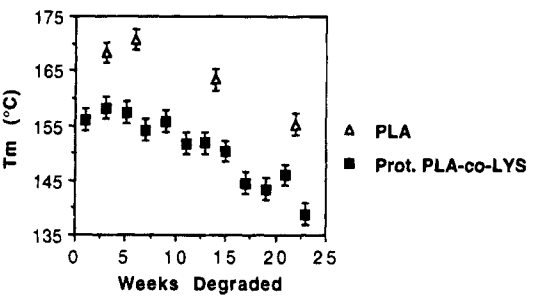


Figure 6. Melting temperatures for Prot. PLA-*co*-Lys and PLA.

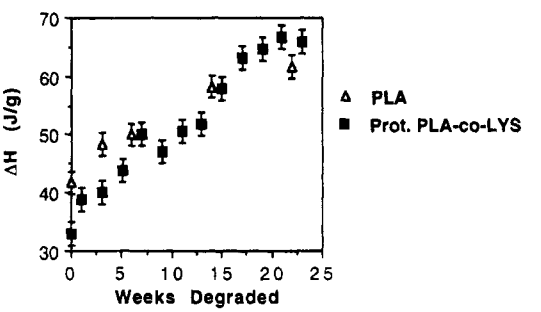


Figure 7. Heat of fusion for Prot. PLA-*co*-Lys and PLA.

values for PLA in Figures 4 and 5. The heat of fusion is directly proportional to the percent crystallinity of the polymer. A larger heat of fusion indicates a higher crystallinity. Two trends were observed. As the degradation proceeds, the crystallinity increases for both samples, while the melting temperatures decrease. Thus, it appears that the amorphous regions are degraded and eliminated first, leaving behind a more crystalline material as expected.[28,30,32-34] The lower melting temperatures indicate that the crystallites that remain become smaller and/or have more defects. The same trends were observed for the Prot. PLA-*co*-Lys as shown in Figures 6 and 7.

The release of lactic acid was measured by an enzymatic assay. Figure 8 shows the amounts of lactic acid released for the different sample types. In general, the release of lactic acid correlates well to the loss of

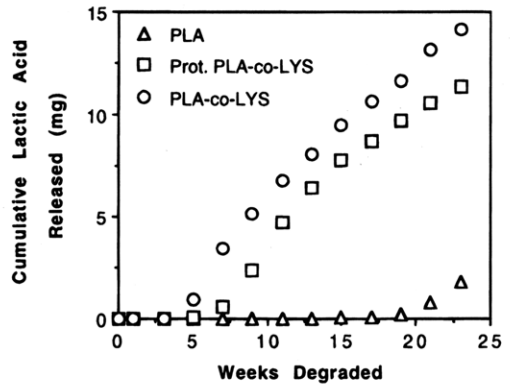


Figure 8. Lactic acid release.

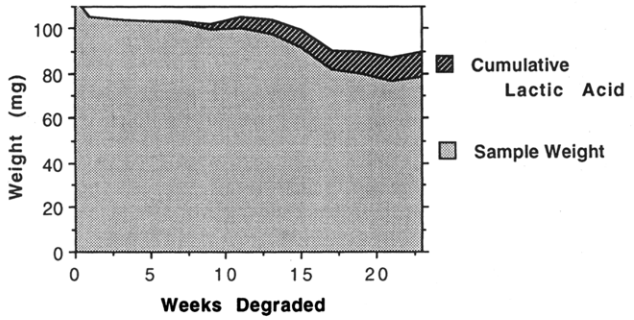


Figure 9. Area comparison of weights for Prot. PLA-*co*-Lys.

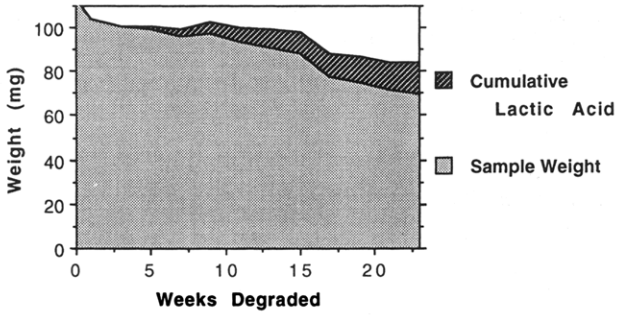


Figure 10. Area comparison of weights for PLA-*co*-Lys.

mass. However, the amount of lactic acid detected by the assay only accounts for about half of the mass loss. This difference is more clearly illustrated in Figures 9 and 10. The total area under the combined curves decreases since the lactic acid assay does not detect 100% of the lactic acid released. The degraded polymer chains probably solubilize before reaching the monomeric state, and one study has detected the presence of the dimer of lactic acid, lactyl lactate, in the degradation buffer.[35] It is suspected that the enzymatic assay does not respond to any form of lactic acid other than the monomeric unit. Therefore, the difference between the lactic acid release data and the weight losses of the samples suggests that monomeric lactic acid is not the only degradation product. Dimers and other short oligomers are released into the buffer as well.

The copolymer composition was determined by amino acid analysis. Additional copolymer composition data was obtained from ^{1}H NMR for the Prot. PLA-*co*-Lys samples. These data are summarized in Table 4. The lysine content decreases as the polymer degrades, with the greatest change occuring as the molecular weight values asymptote to their lower limits around week 15. Two factors contribute to this decrease in lysine content. First, lysine is likely to be concentrated in the amorphous regions. Since these regions degrade more quickly

Table 4. Copolymer Composition of Hydrolytic Degradation Study Samples

sample	time (weeks)	lysine (%) AAA[a]	lysine (%) ^{1}H NMR[b]	sample	time (weeks)	AAA lysine (%)[a]
protected PLA-*co*-Lys	0.0	0.79	1.5	PLA-*co*-Lys	0.0	0.73
	1.0	0.60	1.5		1.0	0.60
	3.0	0.79	1.6		3.0	0.72
	5.0	0.93	1.5		5.0	0.59
	7.0	1.04	1.5		7.0	1.01
	9.0	0.87	1.5		9.0	0.90
	11.0	0.83	1.5		11.0	0.74
	13.0	1.30	1.5		13.0	0.44
	15.0	0.81	1.2		15.0	0.50
	17.0	0.49	0.9		17.0	0.28
	19.0	0.52	0.9		19.0	0.21
	21.0	0.43	0.9		21.0	0.33
	23.0	0.42	0.9		23.0	0.30

[a] Determined by amino acid analysis. Lysine content, not mol % **5**. [b] Determined by ^{1}H NMR. Lysine content, not mol % **5**.

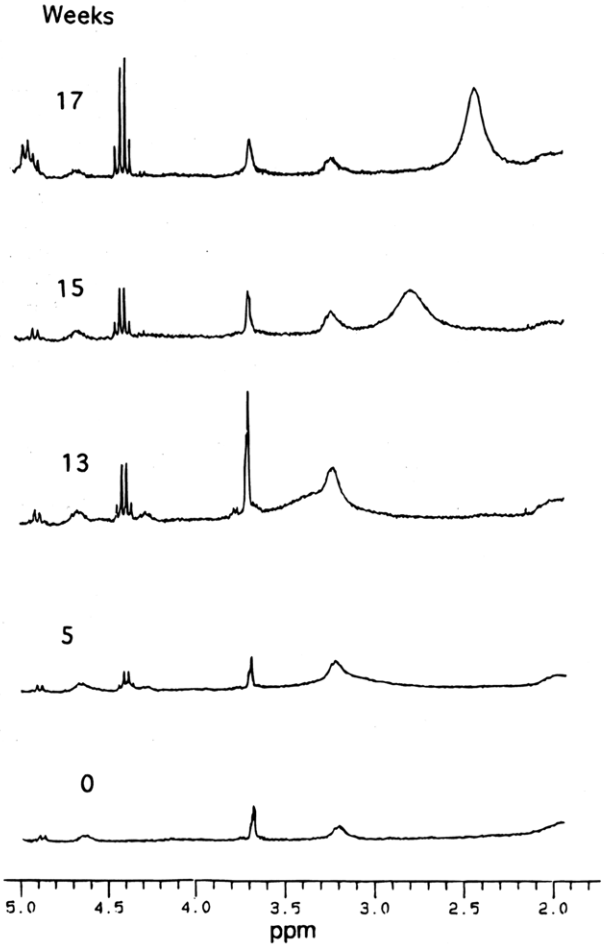


Figure 11. ^{1}H NMR of Prot. PLA-*co*-Lys degradation study samples.

and are removed, the overall lysine content decreases. Second, oligomers containing lysine will be able to dissolve at a higher molecular weight due to their greater hydrophilicity compared to oligomers containing only lactic acid. Therefore, oligomers containing lysine will be removed from the device more quickly than those with few or no lysine units.

Finally, the ^{1}H NMR spectra shown in Figure 11 reveal some interesting changes between weeks 13 and 17 for the Prot. PLA-*co*-Lys sample. A very broad peak develops around 3.3 ppm by week 13. Over the next 4

weeks, this peak becomes more narrow and migrates to 2.38 ppm. After week 17, the peak remains unchanged. The PLA-*co*-Lys samples exhibited similar changes over the same time period. The decrease in the lysine content coincides with the changes in the ^{1}H NMR spectra. The extra peak probably develops due to the increase of hydroxy end groups that occurs as the molecular weight decreases. The change in position may be due to the increasing concentration of hydroxy groups in the NMR test solution since a similar sample weight was used for each analysis. Once the molecular weight levels off, the concentration of hydroxy groups in the NMR test solution remains fairly constant. Therefore, the hydroxy peak stops shifting. The carboxylic acid end group was not detected, indicating that the carboxylic acid may not be protonated. A quartet also develops at 4.35 ppm which can be attributed to the C*H* of the end group.

Conclusions

Biodegradable polymers containing reactive functional groups are an important class of biomaterials. We have successfully synthesized poly(lactic acid-*co*-lysine), which is a biodegradable copolymer that contains a reactive primary amino group on the side chain of lysine. Although the bulkiness of the protected lysine residue apparently decreased the polymerizability of **5** compared to lactide, lysine was successfully incorporated into the copolymer at biologically significant quantities. At the same time, sufficiently high molecular weights were obtained such that the material could be easily processed into useful devices. The degradability of the resulting copolymers was shown to be faster than PLA due mainly to the disruption of crystallinity by the lysine residues.

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