Synthesis of Poly(L-lactic acid-*co*-L-lysine) Graft Copolymers

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Introduction

Poly(glycolic acid), poly(lactic acid), and their copolymers are synthetic polyesters that have been approved by the FDA for certain uses and investigated for many applications.[1−3] However, the cellular response to these polymers (e.g., adhesion and growth of cells) cannot be controlled or modified through changes in the polymer structure, because these polymers do not possess functional groups (other than end groups) that can allow chemical modification to change their properties, thereby limiting the applications of these polymers.

To develop biodegradable polymers that can be modified to tailor their properties for use in a range of applications, while retaining the beneficial properties of the biodegradable polyesters, Barrera et al. synthesized a copolymer (pLAL) (Figure 1) composed of L-lactic acid units and approximately 2% N^{ϵ}-(carbobenzoxy)-L-lysine (Z-L-lysine) units.[4] Random incorporation of lysine into the polymer backbone provides sites along the polymer chain that can be chemically modified through reaction at the ϵ-amine group of the lysine units (after deprotection) to introduce a variety of functionalities onto the polymer backbone. Potential functionalities include RGD (arginine−glycine−aspartic acid) containing peptide sequences[5] or growth factors to improve polymer−cell interactions. In addition to the versatility of these polymers, their biodegradability allows them to serve their purpose and then degrade into lactic acid and lysine, components that can be processed by the body.

The greatest limitation in the copolymers developed by Barrera is that only 2% lysine can be incorporated into the pLAL backbone. In many applications, the concentration of the functional molecules attached to the polymer must be much higher than the 2% will allow. To increase the lysine content of the copolymers, a strategy of using the existing backbone lysine groups as initiating sites for the growth of poly(lysine) side chains was employed. In this paper we report the synthesis of poly(L-lactic acid-*co*-L-lysine) comblike graft copolymers by the ring-opening polymerization of N^{ϵ}-(carbobenzoxy)-L-lysine *N*-carboxyanhydride from the backbone of pLAL and their characterization by elemental analysis, ^{1}H NMR, IR, and GPC. These new polymers provide up to a 35-fold increase in the lysine content, thereby greatly increasing the amount of functional molecules that can be attached. In addition, this system provides the ability to vary the number of reactive lysine sites over a broad range in a well-controlled manner.

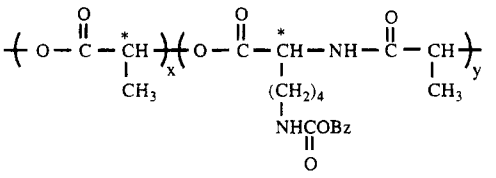

Figure 1. Poly(L-lactic acid-*co*-Z-L-lysine). Removal of the Z protecting group yields the free ϵ-amine that can be utilized for further chemical modification.

Experimental Section

Materials. THF and dioxane were distilled from sodium and stored over a sodium−potassium alloy in the drybox. DMF was stirred overnight over KOH and then distilled from CaO_2. CH_2Cl_2 and $CHCl_3$ were refluxed over CaH_2 and then distilled onto and stored over CaH_2. All other solvents were used as received. Z-L-lysine (Sigma) and triphosgene (Aldrich) were stored in the freezer in the drybox and used as received. HBr (Aldrich, 30 wt % in acetic acid) was used as received. *N,N*-Diisopropylethylamine (Aldrich) was stored over molecular sieves.

Equipment. Reactions were set up or run in a Vacuum Atmospheres drybox (Model HE-43-2). NMR spectra were collected on a Bruker AC250 FTNMR in $CDCl_3$, DMF-d_7, DMSO-d_6, or mixtures of these solvents. IR spectra were collected on a Nicolet 550 FTIR as KBr pellets. GPC studies were conducted in DMF (Phenogel linear column; Phenomenex) or $CHCl_3$ (PLGel guard, linear, and 1000-Å columns in series; Polymer Labs) using a Perkin-Elmer 250 pump and Model LC30 differential refractive index detector. Molecular weights were calculated based on polystyrene standards (Polysciences). Elemental analysis was performed by Quantitative Technologies, Inc., Whitehouse, NJ.

Synthesis of Z-L-lysine *N*-Carboxyanhydride (Z-LYSNCA). According to a procedure by Daly,[6] 3 equiv of triphosgene in THF was added to a slurry of Z-L-lysine in THF at 60 °C. After 3 h, excess hexane was slowly added to precipitate Z-LYSNCA. The mixture was stored overnight in a freezer in the drybox and vacuum filtered to yield 85% Z-LYSNCA, mp 100−101 °C. Anal. Calcd (Found): C, 58.73 (58.82); H, 5.92 (5.88); N, 9.03 (9.15).

Synthesis of poly(L-lactic acid-*co*-Z-L-lysine) Graft Copolymers. Linear poly(L-lactic acid-*co*-L−lysine) with up to 2% lysine content was synthesized as previously described by Barrera.[4] PLAL was deprotected by stirring a slurry of the polymer in HBr/HOAc under argon for 30−60 min. The polymer was then washed with ether and methanol and collected via vacuum filtration. It was then redissolved in $CHCl_3$, neutralized with excess *N,N*-diisopropylethylamine, precipitated from methanol, and dried to yield pLAL with free ϵ-amine groups. Analysis of the polymer by ^{1}H NMR shows the disappearance of the Z-phenyl peak at 7.35 ppm to indicate deprotection is complete.

In the drybox, deprotected pLAL (300 mg; 0.02 mmol of lysine) was dissolved in 5 mL of dioxane, and then a dioxane solution of Z-LYSNCA (64 mg in 3 mL of dioxane; 0.21 mmol) was added. The graft copolymerization mixture was brought out of the drybox, purged with argon throughout the reaction or equipped with a Drierite drying tube, and stirred at room temperature for 2 days. The graft copolymer was precipitated by slowly adding a large excess of methanol, vacuum filtered, and dried under vacuum at room temperature to yield pLAL−LYS in 65% yield.

Results and Discussion

The ring-opening polymerization of amino acid *N*-carboxyanhydrides (NCA's) can be initiated by nucleophilic initiators such as amines, alcohols, and water.[7−9] The primary amine-initiated ring-opening polymeriza-

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Scheme 1

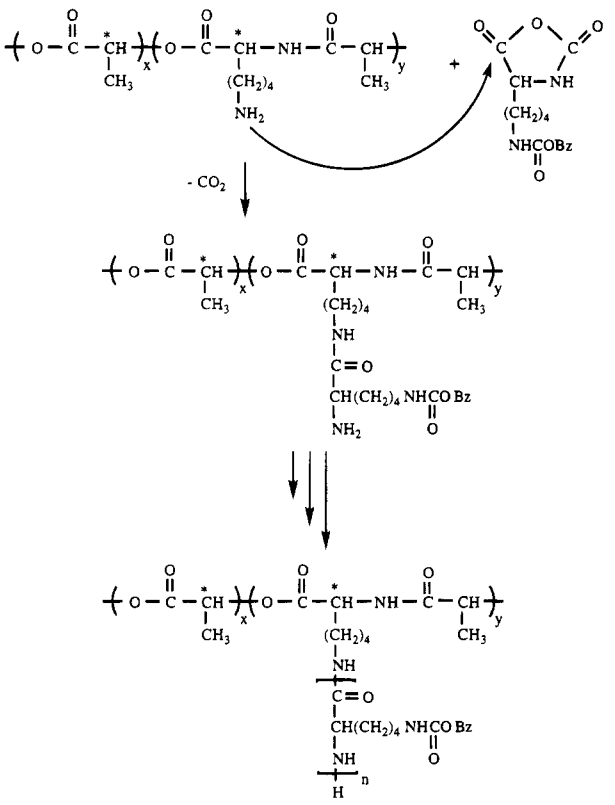

Table 1. Elemental Analysis (C, H, N) and Lysine Content of Copolymers

				lysine (%)	
copolymer	% C	% H	% N	actual	theor[c]
pLAL	48.77	5.45	0.28	0.7	
pLAL-LYS[a]	51.50	6.03	2.40	7	8
pLAL-LYS[b]	56.84	6.33	5.57	23	48
pLAL-LYS[a]	62.03	6.89	9.64	72	81

[a] Copolymerization solvent: dioxane, M_n(pLAL) = 5500. [b] Copolymerization solvent: DMF/CH_2Cl_2, M_n(pLAL) = 8600. [c] See text for details on the determination of the theoretical lysine content.

tion of NCA's, although not a truly "living" process, allows good control over the degree of polymerization when the monomer to initiator ratio (M/I) is less than 150.[7-9]

The free ϵ-amine groups of the deprotected lysine units in the backbone of pLAL have been utilized to initiate the ring-opening polymerization of Z-LYSNCA to yield graft copolymers composed of a poly(L-lactic acid) backbone and poly(Z-L-lysine) side chains. The grafting process is presented in Scheme 1. The nucleophilic primary ϵ-amine of the lysine side group attacks C-5 of Z-LYSNCA, leading to ring opening and formation of the amino acid amide, along with the evolution of CO_2. Propagation takes place via further attack of the amine group of the amino acid amides on subsequent Z-LYSNCA molecules. Graft copolymers (pLAL–LYS) have been synthesized with lysine contents in the range of 7–72% (Table 1). The degree of polymerization of the poly(Z-L-lysine) side chains and the corresponding lysine content in the graft copolymers can be controlled by changing the M/I ratio for the NCA polymerization—that is, changing the ratio of Z-LYSNCA to lysine ϵ-amine groups of pLAL. For this work, poly(Z-L-lysine) degrees of polymerization ranging from approximately 10 to 100 were used. The solubilities of the graft copolymers differ from that of pLAL. pLAL is soluble

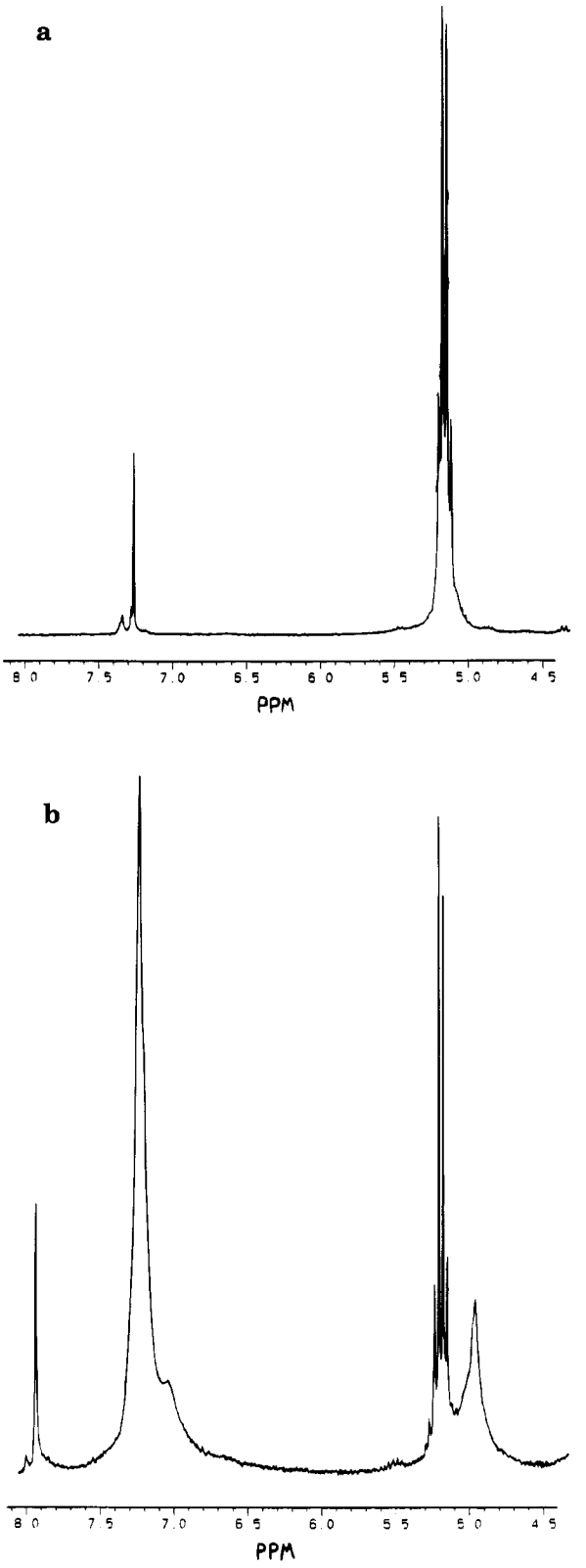

Figure 2. ^{1}H NMR spectra: (a) poly(L-lactic acid-*co*-Z-L-lysine) linear copolymer with 1% lysine content in $CDCl_3$; (b) poly(L-lactic acid-*co*-Z-L-lysine) graft copolymer with 10% lysine content in DMF-d_7.

in less polar solvents such as $CHCl_3$ and CH_2Cl_2, whereas the graft copolymers are only soluble in more polar solvents such as DMF, DMSO, and hexafluoroisopropyl alcohol (HFIP). The range and control of the lysine content (i.e., the number of reactive sites) in these copolymers and the corresponding level of functional molecules that can be attached to the copolymers is 35

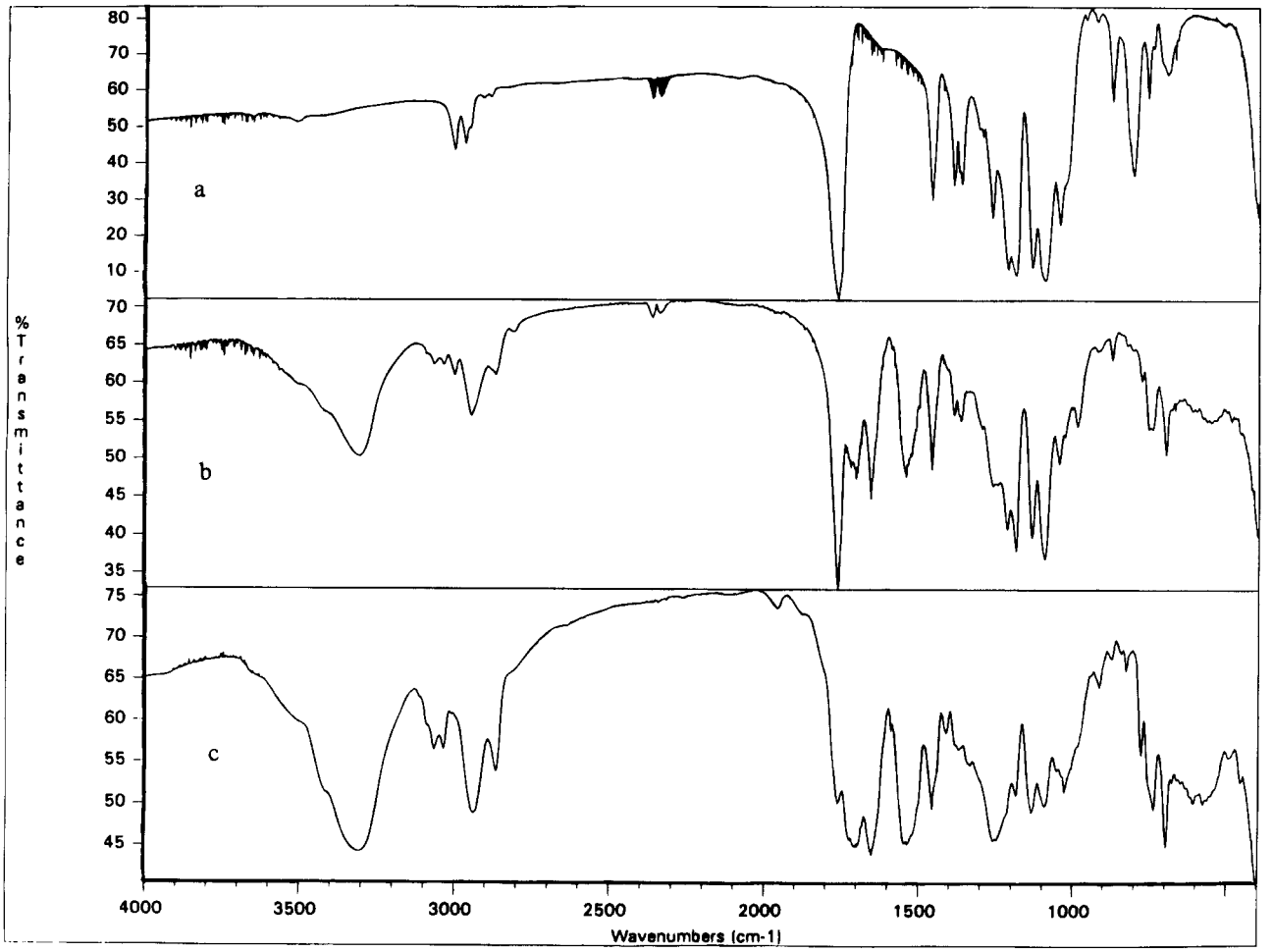

Figure 3. IR spectra (KBr pellets): (a) poly(L-lactic acid-*co*-Z-L-lysine) linear copolymer; (b) poly(L-lactic acid-*co*-Z-L-lysine) graft copolymer with 7% lysine content; (c) poly(L-lactic acid-*co*-Z-L-lysine) graft copolymer with 72% lysine content.

times greater than the previously synthesized pLAL containing only 2% lysine in the polymer backbone.

Elemental Analysis. Table 1 contains the elemental analysis data for a series of polymers and the corresponding lysine content of each polymer. A representative pLAL sample was analyzed and shown to contain 0.7% lysine. The number of lysine units per pLAL chain varies depending upon the molecular weight of the copolymer. The number-average molecular weights (M_n) of pLAL used for this work ranged from 6000 to 25 000, providing between 1 and 7 lysine units per pLAL backbone. The remaining entries in Table 1 are for graft copolymers with varying lysine content. As can be seen from the data, the content of the graft copolymers has been varied in a controlled fashion from 7 to 72%. For the graft copolymerizations run in dioxane (entries 2 and 4), the actual values of lysine incorporated in the copolymers, 7% and 72%, are close to the theoretical values of 8% and 81%, respectively, calculated from the ratio of Z-LYSNCA to lysine ϵ-amine groups of pLAL. Entry 3 is a graft copolymer synthesized in a DMF/CH_2Cl_2 solvent mixture. The dioxane polymerizations remained clear throughout the polymerization, whereas some cloudiness appeared in the polymerizations run in DMF or DMF/CH_2Cl_2. In addition, homopolymerization of Z-LYSNCA took place in the latter systems even though the DMF was purified. The removal of homopoly(Z-L-lysine) from the graft copolymer can be accomplished via fractional precipitation; however, this is a time-consuming process. No homopolymerization occurred in dioxane; therefore, it is the preferred solvent to perform the graft copolymerizations. Because of the consumption of Z-LYSNCA by its homopolymerization, the amount of lysine (23%) incorporated in entry 3 is far less than the 48% calculated from the M/I ratio.

NMR. A portion of the ^1H NMR spectrum of pLAL with a lysine content of approximately 1% is shown in Figure 2a. The peak of the methine proton of the lactic acid repeat units at 5.15 ppm is much larger than the phenyl peak of the Z protecting group of the lysine units at 7.35 ppm. Also seen is the chloroform solvent peak at 7.26 ppm. The spectrum of a pLAL–LYS graft copolymer in DMF-d_7 with 7% lysine content (Figure 2b), however, has a much larger phenyl peak. This peak has shifted 0.15 ppm upfield from the value seen in Figure 2a due to the change of solvent. The lysine content is now high enough that the benzyl CH_2 peak of the Z protecting group can be seen at 4.9 ppm. Due to solvent effects, care must be taken when analyzing the integral values of the NMR spectra.[10]

IR. Infrared spectroscopy was also used to investigate the composition of the graft copolymers. Samples were analyzed as KBr pellets. Figure 3a contains the IR spectrum of poly(L-lactic acid). The peaks of interest are the ester peak at 1759 cm^{-1} and aliphatic peaks at 2999 and 2964 cm^{-1}. In the IR spectrum of a graft copolymer with 10% lysine content (Figure 3b), new peaks can be seen at 3303 cm^{-1}, 1653 cm^{-1} (amide I), and 1531 cm^{-1} (amide II) corresponding to the poly(Z-L-lysine) backbone and 1700 cm^{-1} from the carbamate peak of the Z protecting group. These peaks are

proportionally stronger in the IR spectrum of a graft copolymer with 72% lysine content (Figure 3c).

Molecular Weight Determination. The molecular weights of the graft copolymers can be calculated using the elemental analysis data and the molecular weight of the linear pLAL used for grafting from the following equations:

$$\text{MW graft copolymer} = M_n(\text{pLAL}) + \text{MW(pZLYS)} \quad (1)$$

where M_n(pLAL) is the number-average molecular of pLAL determined by GPC and MW(pZLYS) is the molecular weight of the pZLYS chains calculated from eq 2.:

$$\text{MW(pZLYS)} = \frac{M_n(\text{pLAL})}{2800/262x - 1} \quad (2)$$

where x is the value of % N obtained from elemental analysis.

As an example, the MW of the graft copolymer can be calculated for a graft copolymer with 72% Z-lysine content. The corresponding % N from elemental analysis is 9.64, and M_n(pLAL) was determined by GPC to be 5500. Therefore, from eq 2 the MW of the pZLYS chains is 50 640, and the MW of the graft copolymer from eq 1 is equal to 56 140.

GPC has not been used to determine the molecular weights of the graft copolymers. The branching in the graft copolymers along with the interactions between the poly(lactic acid) and poly(Z-lysine) chains will have a strong influence on their hydrodynamic volume; therefore, the use of linear standards such as polystyrene or pMMA will result in inaccurate calculation of the molecular weights of the graft copolymers. GPC is a necessary tool to verify the synthesis of pLAL–LYS that is free of homopoly(Z-lysine) or homo-pLAL contaminants. The presence of a single peak detected by GPC at a retention time different than that of the initial pLAL combined with the detection of lysine by elemental analysis, ^{1}H NMR, and IR proves that lysine is incorporated in the polymer and that a single species, i.e., the graft product, has been prepared.

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(10) The size of the peaks corresponding to the Z groups of the lysine chains varies depending upon the solvent, due to interactions between the solvents and the poly(lactic acid) and poly(Z-lysine) chains. In DMF-d_7 or DMSO-d_6, the copolymer conformation is such that the Z groups are more exposed to the solvents through more favorable interactions with the poly(Z-lysine) chains, resulting in larger lysine peaks relative to the poly(lactic acid) peaks.

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