Synthesis and Characterization of Biodegradable Poly(lactic acid-co-glycine) via Direct Melt Copolymerization

Zhaoyang Wang1*, Xiaona Hou1, Zhengzhou Mao1, Ruirong Ye1 Yangqing Mo1, and David E Finlow2

(1) Department of Chemistry, South China Normal University
Guangzhou-510006, PR China
(2) Shawnee State University, Portsmouth, Ohio, 45662-4344, USA

Received 30 May 2008; accepted 11 October 2008

Instead of the traditional two-step method with cyclic intermediates, a direct reaction of D,L-lactic acid (D,L-LA) and glycine (Gly) as monomers (molar feed ratio LA/Gly: 90/10) and SnO (0.7 wt%) as catalyst is carried out to synthesize a biodegradable poly(lactic acid-co-glycine) [P(LA-co-Gly)] via melt copolycondensation at 170°C, absolute pressure of 70 Pa, for 8 h, following a prepolymerization step. The structure of the copolymer was characterized by FTIR and 1H NMR techniques. The GPC results show that the weight average molecular weight ($M_w$) of the copolymer P(LA-co-Gly) is 3600 g/mol. Under the condition of different molar feed ratios the highest $M_w$ was found to be 5200 with molar feed ratio LA/Gly:98/2. Other characterizations, e.g., intrinsic viscosity, solubility, DSC, and XRD indicated that the copolymer solubility in chloroform and tetrahydrofuran (THF) decreased with the increasing feed charge of Gly and the lowest solubility was found when the molar feed ratio was below LA/Gly:70/30. However, the modified polylactic acid is readily soluble in DMSO. The crystallinity of copolymer gradually disappears as the molar feed ratio of D,L-LA decreases. For LA/Gly ≥ 90/10, the XRD pattern has two diffraction peaks, close to 16.8° and 19.1°, the same position as poly(D,L-lactic acid) (PDLLA). For the soluble P(LA-co-Gly) the intrinsic viscosity, $M_w$ and its dispersity ($M_w/M_n$) decrease gradually with the increase of feed charge of Gly. These results, especially the molecular weight of the copolymer appear to meet the requirements necessary for a drug delivery applications.

INTRODUCTION

Glycine (Gly) is the simplest and one of the natural amino acids in proteins [1]. Its (co)polymers have been widely investigated [2-10]. Especially, since Helder [5,6] used biodegradable poly(lactic acid-co-glycine) [P(LA-co-Gly)] as biomedical material, increasing emphasis has been given to the applications of Gly-modified poly(lactic acid) (PLA) in drug delivery systems [7-10].

The property of the components or monomer units, especially their impact on biosystems, in biodegradable PLA copolymer or its blends is crucial in their performance [11-13]. Gly released from in
vivo degradation of P(LA-co-Gly) is safe and non-toxic. For this reason, Gly is extensively uses as additive in foods, foodstuffs, and cosmetics [1]. More importantly, as an amino acid component of the endogenous antioxidant glutathione (GSH), Gly has important specific protection functions for cells, tissues, and organs, e.g., the protection of PC-12 cells against injury by ATP-depletion [14-18].

Therefore, as a drug delivery material, P(LA-co-Gly) shows great promises. But expensive and time-consuming synthetic procedure of the copolymer has limited its many diverse applications. Typically, P(LA-co-Gly) was synthesized via a two-step method. Cyclic intermediates, such as 6-methyl-2,5-morpholinedione, and lactide were first prepared from Gly, lactic acid (LA), or their derivatives, through difficult multi-step reactions, and then ring-opening polymerization of the cyclic intermediates gave the copolymer P(LA-co-Gly) [7-9].

In recent years, with the improvement of synthetic techniques of PLA using LA as starting material [19-26], great importance has been directed towards the direct copolycondensation of LA with other monomers [27-31]. In order to reduce the synthesis cost of biodegradable P(LA-co-Gly) and extend its application, it is necessary to investigate its direct synthesis by using a novel one-step method instead of the traditional two-step method with lactide as an intermediate.

In fact, other copolymers of α-hydroxy acids (e.g., LA or glycolic acid) and an α-amino acid, including poly(lactic acid-co-α-amino acid) (PLAA) were also obtained through a two-step method and the molecular weight of PLAA has seldom been reported in the published papers. For example, poly(lactic acid-co-aspartic acid) is synthesized via intermediates [(3S)-3-[benzyl ether carbonyl]methyl]morpholine-2,5-diketone and lactide [32] without any information on its molecular weight. In other reports, the number average molecular weight (Mn) of a copolymer synthesized from cyclic intermediates, such as morpholine-2,5-dione, is found to be approximately 3000 g/mol [33-36].

In the present work, in continuation of our previous works on direct melt polycondensation of LA [21, 22, 29, 30] we report for the first time a simple and practical method for the synthesis of P(LA-co-Gly), the direct synthesis via melt copolymerization of Gly and LA (Scheme I). The structure and properties of the obtained polymers are investigated as well. The results show that it is possible to synthesize PLAA directly, especially when the α-amino acid modified PLA is applied in drug delivery systems [37].

**EXPERIMENTAL**

**Materials**

D,L-Lactic acid (D,L-LA) and SnO were purchased from Guangzhou Chemical Reagent Factory, and glycine was purchased from Shanghai Kangda Amino Acid Factory. All other chemicals were commercially available as analytical grades and used without further purification.

**Instrumental Analysis and Measurements**

The intrinsic viscosity ([η]) was determined in an Ubbelohde viscometer with CHCl₃ as the solvent at 25°C. Gel permeation chromatographic (GPC) analysis was performed on a USA Waters 1515 HPLC using THF as the solvent and PS for calibration with a flow velocity 1 mL/min at 40°C. In some of the polymer characterization tests, the insoluble fractions were removed via filtration after the dissolving process.

FTIR spectra were recorded on a German Bruker Vector33 FTIR spectrometer by the KBr salt slice method. ¹H NMR spectra were measured on a German Bruker DRX-400 spectrometer in DMSO-d₆, using TMS as internal reference.

![Scheme I. The synthetic route of P(LA-co-Gly).](image-url)
The thermal properties of the polymer were measured with a USA Perkin-Elmer DSC7 thermal analyzer at a heating rate of 10°C/min under a nitrogen atmosphere (flow velocity 20 mL/min). The crystallinity of the polymer was carried out on a Y-2000 X-ray diffraction (XRD) apparatus (made by China Dandong XRD Apparatus Co., Ltd.) using CuKα radiation with a wavelength of 1.5406×10⁻¹⁰ m, and scanning range 2θ = 1-40°.

**Melt Copolymerization**

A procedure from our previous reports on the melt homo-(or co-) polymerization of LA [21,22,29,30] was adopted. LA and Gly with predetermined molar feed ratios were mixed until a uniform mixture was formed. The mixture was then directly dehydrated for 6 h at 140°C and 4000 Pa in a three-necked flask equipped with a mechanical stirring device and a thermometer. The catalyst, SnO, was then added as 0.7 weight percentage of dehydrated reactants (0.7 wt%). After prepolymORIZATION, melt copolymerization was carried out for 8 h at 170°C under an absolute pressure of 70 Pa. When the reaction was complete, the product was cooled to room temperature, dissolved in CHCl₃ and precipitated in CH₃OH to give a white powder. The resulted polymer was then dried in vacuo to constant weight. The yield was within the range of 20% and 57%, and in most cases it was above 30%.

**RESULTS AND DISCUSSION**

Using inexpensive D,L-LA and Gly as starting materials, some random copolymer P(LA-co-Gly) oligomers with different molar feed ratios (LA/Gly = 98/2, 95/5, 90/10, 80/20, 70/30, 50/50) were synthesized directly via melt copolycondensation. The structure and properties of these P(LA-co-Gly) were characterized by FTIR, ¹H NMR, GPC, DSC, and XRD techniques and viscosity measurements.

**Structural Characterization of P(LA-co-Gly)**

The structural characterization of poly(D,L-lactic acid) (PDLLA) was compared with the product of the direct melt homopolymerization of LA [21,22], P(LA-co-Gly) synthesized with molar feed ratio 90/10 (LA/Gly). It was elucidated that these compounds show similar absorptions in their FTIR spectra, vis-a-vis the absorption of ester carbonyl at 1749 cm⁻¹. The absorptions at 3300 cm⁻¹, 1615 cm⁻¹ and 1545 cm⁻¹ from -CONH- group appeared in the FTIR spectrum of P(LA-co-Gly) while not being observed in that of PDLLA, a strong indication that -CONH- linkages has been introduced into the copolymer.

¹H NMR data of P(LA-co-Gly) with molar feed ratio LA/Gly:90/10 and δ, ppm (DMSO-d₆ as solvent and TMS as internal reference): 1.48 (d, CH₃ in LA chain), 3.95 (m, CH₂ in Gly chain), 5.22 (q, CH in LA chain), 8.52 (d, -CONH-). The chemical shift from -CONH- group in our polymer is 8.52 ppm, very close to the value 8.30 ppm of the poly(D,L-lactic acid-co-L-lysine) random copolymer synthesized via two-step method as reported in the literature [38]. These data from FTIR and ¹H NMR indicate that the direct melt copolycondensation of LA and Gly indeed gave the copolymer P(LA-co-Gly) (Scheme I). This was further proved by the result of GPC.

For the molecular weight determination and molecular weight distribution of the copolymers a GPC curve of P(LA-co-Gly) with molar feed ratio LA/Gly:90/10 was obtained and showed only a single peak. The corresponding weight-average molecular weight (M₀) was 3600 g/mol, number-average molecular weight (Mₙ) was 2900 g/mol, and the polydispersity index (PDI) M₀/Mₙ was 1.24. The monomodal peak (Figure 1) and low PDI (M₀/Mₙ< 2) again indicated that the product of direct melt copolycondensation from two monomers is indeed a copolymer, and not a mixture containing any homopolymer, PDDLA, or polyglycine.
Usually, when the PLA biodegradable polymers were used as drug delivery material, their molecular weights were no more than 30000 [22]. As reported in the literature, the PLAs material with molecular weight of 1800 could be applied in drug delivery, even the PLA polymers with molecular weight of only 900 could be used as drug delivery device [39,40]. The molecular weight of P(LA-co-Gly) synthesized here via direct melt copolycondensation was overwhelmingly higher than 900 g/mol. Therefore, its molecular weight meets the requirement for drug delivery applications.

Thermal Properties and Crystallinity
The thermal properties and crystallinity of P(LA-co-Gly) (molar feed ratio LA/Gly:90/10) were characterized by DSC and XRD techniques, and the data are shown in Table 1. It is obvious that both $T_g$ and $T_m$ of P(LA-co-Gly) are lower than those of PDLLA, indicating that the introduction of the Gly fragments into PLA chain disturbed the regularity. This was further demonstrated by the XRD results.

XRD results show that P(LA-co-Gly) is not totally amorphous. As the PLA chain was the main component of the copolymer, the position of crystalline peak was similar to that of the PDLLA (Table 1). Obviously, P(LA-co-Gly) has a lower crystallinity and the crystallite size is smaller than PDLLA. These facts confirmed that the introduction of the Gly component into PLA chain has altered the regularity of the polymer, but the extent of their effects may be connected with the content of Gly component present in the copolymer.

Studies on Different Molar Feed Ratios
The structural studies on copolymers with different molar feed ratios studied by FTIR and $^1$H NMR analyses showed similar features. However, in case of increased Gly content, more -CONH- linkage was formed and the strength of -CONH- absorption band increased as well. Particularly, for LA/Gly:50/50, the intensity of the absorption from the carbonyl group in -CONH- is stronger than that of the carbonyl group in esters, and the peak position was moved to 1690 cm$^{-1}$ (Figure 2).

Solubility test indicated that the solubility of P(LA-co-Gly) in chloroform and THF decreased as

<table>
<thead>
<tr>
<th>Samples</th>
<th>$T_g$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\theta$ (Degree)</th>
<th>Crystallinity (%)</th>
<th>Crystallite size ($\times10^{-10}$ m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDLLA [22]</td>
<td>54.6</td>
<td>120.0</td>
<td>-16.7</td>
<td>20.8</td>
<td>154.4</td>
</tr>
<tr>
<td>P(LA-co-Gly)</td>
<td>51.1</td>
<td>83.2</td>
<td>-16.7</td>
<td>16.7</td>
<td>137.5</td>
</tr>
</tbody>
</table>

Table 1. The DSC and XRD data of P(LA-co-Gly) synthesized as molar feed ratio 90/10 (LA/Gly).

Figure 2. The infrared spectra of P(LA-co-Gly).
the content of Gly increased, but the modified PLA was readily soluble in DMSO. Therefore, the relationship between $[\eta]$ and $M_\text{w}$ is only applied to the soluble product as shown in Table 2. It was obvious that the $[\eta]$ in DMSO was lower than that in chloroform when the molar feed ratio is the same and decreased also as the content of Gly increased. The results proved that the solubility in DMSO is higher than that in chloroform, and introducing more Gly into the copolymer is not advantageous for obtaining a copolymer with higher $M_\text{w}$.

As shown in Table 2, the homopolymerization of LA is inhibited by Gly, and the greater the content of Gly in feed, the smaller are $[\eta]$, $M_\text{w}$, and $P_{\text{DI}}$ ($M_\text{w}/M_\text{n}$) for the soluble P(LA-co-Gly). In case of LA/Gly:50/50, however, the melt copolycondensation is complicated, because the solubility of the product in CHCl$_3$ and THF is smaller than those with the other feed ratios, and the GPC results showed that the product may contain two polymers with different molecular weights (Table 2). Thus, lower Gly content in the feed is recommended for the synthesis of P(LA-co-Gly) via direct melt copolymerization of LA and Gly.

### Influence on the Thermal Properties

The effects of different molar feed ratios on $T_g$ and $T_m$ of the product is presented in Table 3. The -CONH- linkage is conducive to the formation of interchain hydrogen bonds. With the increase in Gly content of the feed, more -CONH- linkages are introduced into the copolymer, yielding higher $T_g$ values for P(LA-co-Gly). For LA/Gly:50/50, $T_g$ and $T_m$ of the complex product are not observed, suggesting that the molar feed ratio for melt copolycondensation should be more than 70/30 (LA/Gly).

At the same time, the heat of fusion, $\Delta H$, decreased as the number of -CONH- linkages in the

### Table 2. The effects of molar feed ratios on solubility, $[\eta]$, and $M_\text{n}$ of the copolymerization product.

<table>
<thead>
<tr>
<th>LA/Gly</th>
<th>Solubility (mg.mL$^{-1}$)</th>
<th>$[\eta]$ (dL.g$^{-1}$)</th>
<th>$M_\text{w}$</th>
<th>$M_\text{n}$</th>
<th>$M_\text{w}/M_\text{n}$</th>
<th>Peak shape</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHCl$_3$</td>
<td>DMSO</td>
<td>THF</td>
<td>CHCl$_3$</td>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td>98/2</td>
<td>&gt;2.000</td>
<td>&gt;2.000</td>
<td>&gt;4.012</td>
<td>0.9833</td>
<td>0.8693</td>
<td>5200</td>
</tr>
<tr>
<td>95/5</td>
<td>&gt;2.000</td>
<td>&gt;2.000</td>
<td>&gt;4.012</td>
<td>0.9584</td>
<td>0.8074</td>
<td>5100</td>
</tr>
<tr>
<td>90/10</td>
<td>&gt;2.000</td>
<td>&gt;2.000</td>
<td>&gt;4.012</td>
<td>0.7780</td>
<td>0.6959</td>
<td>3600</td>
</tr>
<tr>
<td>80/20</td>
<td>&gt;2.000</td>
<td>&gt;2.000</td>
<td>&gt;4.012</td>
<td>0.7634</td>
<td>0.6217</td>
<td>2900</td>
</tr>
<tr>
<td>70/30</td>
<td>1.328</td>
<td>&gt;2.000</td>
<td>1.616</td>
<td>0.5293</td>
<td>0.4727</td>
<td>2400</td>
</tr>
<tr>
<td>50/50</td>
<td>0.728</td>
<td>&gt;2.000</td>
<td>0.584</td>
<td>0.4543</td>
<td>0.4479</td>
<td>1400</td>
</tr>
</tbody>
</table>

(a) P(LA-co-Gly) was mainly insoluble in CHCl$_3$ and THF. (b) A single peak, weaker than other case, and a smaller, single peak at its right.

### Table 3. The influences of molar feed ratios on $T_g$, $T_m$, and crystallinity of the copolymerization product.

<table>
<thead>
<tr>
<th>LA/Gly</th>
<th>$T_g$ ($^\circ$C)</th>
<th>$T_m$ ($^\circ$C)</th>
<th>Heat of fusion $\Delta H$ (J.g$^{-1}$)</th>
<th>Crystallinity (%)</th>
<th>Crystallite size ($\times 10^{-10}$ m)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$L_{110}$</td>
<td>$L_{020}$</td>
</tr>
<tr>
<td>98/2</td>
<td>46.7</td>
<td>124.8</td>
<td>36.12</td>
<td>27.1</td>
<td>177.2</td>
<td>97.1</td>
</tr>
<tr>
<td>95/5</td>
<td>49.5</td>
<td>110.6</td>
<td>33.06</td>
<td>25.7</td>
<td>159.0</td>
<td>98.9</td>
</tr>
<tr>
<td>90/10</td>
<td>51.1</td>
<td>83.2</td>
<td>11.70</td>
<td>16.7</td>
<td>137.5</td>
<td>56.0</td>
</tr>
<tr>
<td>80/20</td>
<td>54.7</td>
<td>112.0 a</td>
<td>ND b</td>
<td>ND b</td>
<td>ND b</td>
<td>Amorphous</td>
</tr>
<tr>
<td>70/30</td>
<td>65.5</td>
<td>ND b</td>
<td>ND b</td>
<td>ND b</td>
<td>ND b</td>
<td>Amorphous</td>
</tr>
<tr>
<td>50/50</td>
<td>ND b</td>
<td>ND b</td>
<td>ND b</td>
<td>ND b</td>
<td>ND b</td>
<td>Amorphous</td>
</tr>
</tbody>
</table>

(a) Only the temperature of initial decomposition was investigated after $T_g$. (b) Not detected.
copolymer increased. This also indicates that the complexity of the product is related to the introduction of -CONH- linkages. In other words, the prepared P(LA-co-Gly) should be a random copolymer, which is also implied by the XRD results (Table 3).

**Influence on Crystallinity**

The heat of fusion, $\Delta H$, of polymer P(LA-co-Gly) reflects its crystallinity to a degree, and its relationship to molar feed ratio is in accordance with the XRD results. Only for LA/Gly $\geq$ 90/10 the XRD pattern gives two diffraction peaks at positions close to 16.8° and 19.1°, the same positions as those of PDLLA [22]. Meanwhile, the crystallinity decreased with the increase of Gly in the feed. These indicate that increasing the number of -CONH- linkages in the copolymer, especially when LA/Gly $\leq$ 80/20 creates a less-ordered structure and crystallization becomes more difficult. However, for LA/Gly $\geq$ 95/5, the formation of hydrogen bonds between the chains by the lower number of -CONH- linkages possesses P(LA-co-Gly) with higher crystallinity and heat of fusion compared to PDLLA (20.8%, and 17.12 J.g$^{-1}$, respectively [22]).

Similarly, the crystallite size varied with the content of Gly in feed. For Gly content in feed $\leq$ 5 mol%, the lower number of -CONH- linkages in the modified PLA is in favour of crystallization for the copolymer, because hydrogen bonds facilitate the alignment of the polymer chains, yielding larger crystallite size, higher crystallinity, and larger heat of fusion than PDLLA (Tables 1 and 3). For Gly content in feed $\geq$ 10 mol%, excess number of -CONH- linkages is formed and resulted in a more complex structure and therefore the alignment of the polymer chains is disturbed. This increasing complexity gradually became the dominant factor with increase in Gly content of the feed, yielding smaller crystallite size, lower crystallinity and smaller heat of fusion than PDLLA (Tables 1 and 3).

The crystallinity of polymers has an important effect on their physical and biological properties, especially their degradability which is crucial for biomaterials. This investigation showed that it would be essential to control the feed ratio of Gly content to obtain a copolymer with suitable component of Gly units and thus introduce good biological properties to the copolymers.

**CONCLUSION**

In summary, it is possible to synthesize P(LA-co-Gly) simply by employing LA and Gly directly as starting materials, instead of a two-step method using the intermediates, 6-methyl-2,5-morpholinedione and lactide. The structure and properties of the copolymer have been investigated by FTIR, $^1$H NMR, GPC, DSC, and XRD techniques and solubility and intrinsic viscosity determinations. P(LA-co-Gly) with different molar feed ratios were synthesized, and the highest was 5200 when the molar feed ratio LA/Gly was 98/2. With the increase in Gly content the solubility, intrinsic viscosity, $M_n$, and crystallinity decreased gradually and the structure of the product became more complex. This novel method is simple, practical, and economical. Considering all kinds of factors, especially the solubility of the product, lower Gly feed charge is favourable and the properties, especially the molecular weight of the copolymer is likely to meet the requirements for a drug delivery material.

**ACKNOWLEDGEMENTS**

The authors are grateful to the Natural Science Foundation of Guangdong Province (grant number 5300082) and the Natural Science Foundation of China (grant number 20772035) for the financial support of this work.

**REFERENCES**


