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Synthesis and characterization of a functionalized biodegradable copolymer: poly(L-lactide-*co*-*RS*-β-malic acid)

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Abstract

The ring-opening copolymerization of L-lactide (L-LA) and *RS*-β-benzyl malate (MA) was performed in the presence of stannous octoate. Copolymers were successfully synthesized using different feeding doses. ^{1}H NMR analysis revealed that the compositions of the copolymers with high MA contents were similar to the feeding doses. GPC measurements showed that the molecular weight of the copolymers decreased as the MA content increased. The hydrophilicity of these copolymers was remarkably improved after they were hydrogenolyzed over palladium on charcoal, which removed the pendant benzyl groups. These de-protected copolymers had higher glass transition temperatures (T_g) than both the corresponding protected copolymers and the PLLA homopolymer due to the formation of intermolecular hydrogen bonds between the pendant carboxyl groups. The morphology of the copolymers changed from crystalline to amorphous with increasing *RS*-β-malic acid content.

Keywords: Poly(L-lactide-*co*-*RS*-β-malic acid); Poly(L-lactide-*co*-*RS*-β-benzyl malate); Biodegradable polymer

1. Introduction

Poly(α-hydroxyl acid)s such as PLA and PGA have been widely used as biomedical materials due to their excellent mechanical properties, low immunogenicity and good biocompatibility. Poly(α-hydroxyl acid)s have been fabricated into scaffolds for cell attachment and proliferation in tissue engineering. However, the affinity between the poly(α-hydroxyl acid)s scaffolds and the cells was not good due to the lack of natural recognition sites for the cells on the surface of the polymers. Many efforts have been made to improve the cell affinity of poly(α-hydroxyl acid)s. Functional groups such as amino and carboxyl groups could provide recognition sites for cell attachment. In order to obtain biodegradable polymers with functional pendant groups, some researchers have carried out the copolymerization of α-hydroxyl acids with multifunctional substances, such as the amino acids [1–5]. Vert [6–10] and Ouchi [11] synthesized poly(β-malic acid) and poly(α-malic acid), which are biodegradable polymers. These poly(malic acid)-type polymers are attractive biomedical materials with functional pendant groups [12–18]. However, the application of poly(malic acid)s has limitations because of the long complex synthetic route, the low yields and the low molecular weights of the polymers [19,20]. The copolymerization of malic acid and α-hydroxyl acids appeared to be a good way to resolve these problems. Kimura et al. [21–23] synthesized a cyclic diester monomer consisting of glycolate and benzyl-α-L-malate units and copolymerized it with L-lactide to obtain biodegradable polymers with carboxyl groups. Unfortunately, the synthesis of the cyclic diester monomer, cyclo(Glc-Malatone), was very difficult and the contents of α-malic acid in copolymers was very low (below 10 mol%).

In the present paper, two different monomers (L-lactide and *RS*-β-benzyl malate) were copolymerized by ring-opening polymerization. Poly(L-lactide-*co*-*RS*-β-malic acid) was obtained by removing the benzyl groups. The hydrophilicity, molecular weight and thermal properties of both the protected and de-protected copolymers were determined in detail.

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2. Experiments

2.1. Materials

RS-bromosuccinic acid and benzyl alcohol were purchased from Beijing chemical reagent company. *RS*-bromosuccinic acid was recrystallized in acetonitrile. Benzyl alcohol was distilled. L-lactide was purchased from Purac (The Netherlands) and recrystallized in toluene and ethyl acetate. Stannous octoate, trifluoroacetic acid anhydride (TFAA) and palladium on charcoal (Pd/C) were purchased from Aldrich and used without purification. Solvents such as tetrahydrofuran (THF), 1,4-dioxane and ethyl ether were dried with sodium and distilled. Acetonitrile and chloroform were dried with CaH_2 and distilled. Benzene was used as received.

2.2. Measurement

^{1}H NMR spectra were recorded on a Bruker DMX-300 with tetramethylsilane as the internal standard and acetone-d_6 as the solvent. FTIR spectra were recorded on PE 2000 spectrometer. DSC spectra were measured by METTLER TOLEDO STAR^e System under the heating rate of 10 °C min^{-1} and a nitrogen atmosphere. GPC was performed on Waters 510 with Shodex KF-800 columns, and the data processing software was Waters Millennium32. Samples were measured at 35 °C with chloroform as eluent at a flow rate of 1.0 ml/min. The molecular weight was calibrated relative to polystyrene standards. Water contact angle of copolymer films were measured by Face contact angle meter (Kyowa Kaimen Kagaku Co., Ltd).

2.3. Monomer synthesis

2.3.1. Mixture of RS-3-benzyloxycarbonyl-3-bromopropanoic acid and RS-2-bromo-3-benzyloxycarbonylpropanoic acid

RS-bromosuccinic acid (19.7 g, 0.1 mol) was dried under vacuum for 2 h. Twenty-five milliliters of THF was added under N_2 atmosphere, and 20 ml (0.14 mol) of TFAA was added in drops. The mixture was stirred and kept in ice bath. When the mixture became clear, the solvent was evaporated under vacuum and a kind of pale brown oil was left over. Distilled benzyl alcohol (10.8 g, 0.1 mol) was added immediately and the system was stirred at 45 °C for 12 h to give a bright yellow monoester mixture. The mixture was dissolved in 100 ml of ethyl ether, washed with 100 ml of water for three times and dried over $MgSO_4$/decolorizing charcoal. After filtration, the solvent in filtrate was evaporated to get white oil product.

Characteristics of monoester mixture: *w* (weight) = 30.0 g; yield = 98%. ^{1}H NMR (300 MHz, CD_3COCD_3, δ ppm): 2.93–2.95 (d, 2H), 4.06–4.10 (m, 1H), 5.04 (s, 2H), 7.25 (s, 5H). FTIR spectrum (cm^{-1}): 3035 (ν CH of aromatic ring), 1744 (ν C=O of $COOB_Z$), 1719 (ν C=O of COOH). 751 and 697 (δ CH of aromatic ring).

2.3.2. RS-4-benzyloxycarbonyl-2-oxetanone

Thirty grams of monoester mixture was put in a beaker. A solution of 2N NaOH was added slowly to adjust to the pH 7.2. The solution was heated to 45 °C and 150 ml of benzene was added. Being stirred vigorously for 3 h at 45 °C, the organic phase was washed with 150 ml of 5% $NaHCO_3$ aqueous solution twice and distilled water till neutrality, then it was dried over $MgSO_4$. After filtration, the benzene was evaporated to give 5.89 g of crude *RS*-β-benzyl malate. The crude product was purified by chromatography on silica gel.

Characteristics of purified product: *w* (weight) = 2.57 g, yield = 12.5% (respect to *RS*-bromosuccinic acid), bp = 115–117 °C, under 2×10^{-2} mm Hg. Elementary analysis: found: C = 64.02%; H = 4.89%; O = 31.09%; calculated: C = 64.08%; H = 4.85%; O = 31.07%. ^{1}H NMR (300 MHz, CD_3COCD_3, δ ppm): 3.61–4.12 (m, 2H), 5.08–5.17 (dd, 1H), 5.28 (s, 2H), 7.41 (s, 5H). FTIR spectrum (cm^{-1}): 1848 (ν C=O of lactone), 1744 (ν C=O of $COOB_Z$).

2.4. Copolymerization

Prescribed amount of L-lactide and *RS*-β-benzyl malate with 0.1 wt% (respect to the total weight of monomer mixture) stannous octoate were put into polymerization tube together with a magnetic stirrer. The polymerization tube was purged with nitrogen for three times, sealed under vacuum and put into oil bath at 110 °C for 108 h. The product was dissolved in chloroform and precipitated into a large amount of ethyl ether. The poly(L-lactide-*co*-*RS*-β-benzyl malate) precipitate was dried under vacuum at room temperature for two days.

2.5. Hydrogenolysis

one gram of poly(L-lactide-*co*-*RS*-β-benzyl malate), 50 ml of 1,4-dioxane and 0.2 g of palladium on charcoal were put into a 500 ml flask together and stirred vigorously. Deoxygenation was performed by hydrogen bubbling for 24 h. After filtration, the solution was condensed and dropped into a large amount of ethyl ether. White poly(L-lactide-*co*-*RS*-β-malic acid) precipitate was dried under vacuum at room temperature for two days.

2.6. Preparation of polymer film

A 5 wt% solution of poly(L-lactide-*co*-*RS*-β-malic acid) in 1,4-dioxane was prepared and cast onto a glass plate. Polymeric films were obtained after the solvent evaporated in air and further dried under vacuum to remove any residual solvent. For comparison, films of PLLA and

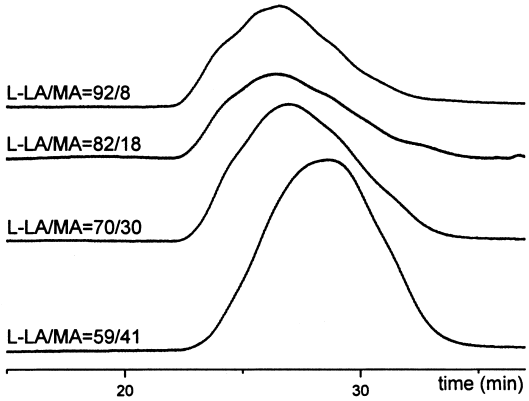


Fig. 1. GPC curves of poly(L-lactide-*co*-*RS*-β-benzyl malate).

protected copolymer were also prepared by the same method.

3. Results and discussion

3.1. Copolymerization of L-lactide and RS-β-benzyl malate

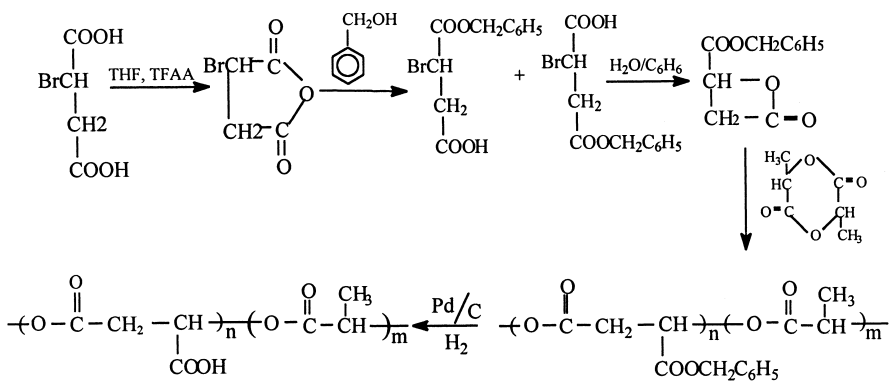
In tissue engineering, the affinity between hydrophobic PLLA scaffolds and cells is not good due to the lack of functional groups, which serve as cell recognition sites for cell attachment. In this study, water-soluble malic acid molecules were introduced into PLLA to functionalize PLLA and promote its hydrophilicity. This copolymer could be a new potential tissue engineering scaffold material. The synthetic route of poly(L-lactide-*co*-*RS*-β-malic acid) is shown in Scheme 1. Two different cyclic monomers of L-lactide and *RS*-β-benzyl malate were copolymerized by ring-opening polymerization in the presence of stannous octoate, which was an efficient initiator for the poly(α-hydroxyl acid)s. All the copolymers were obtained as white powders in high yield (>80%). The benzyl ester could be easily removed by hydrogenolysis using palladium on charcoal as the catalyst, and the composition of the functional groups in the copolymers could be controlled by adjusting the MA content in the feeding dose. This synthetic route was simple and relatively short compared to the previous route used to synthesize copolymers containing malic acid [21].

Table 1
GPC results of poly(L-lactide-*co*-*RS*-β-benzyl malate)

Sample number	MA component (%)		Copolymer			
	Feed	Copolymer[a]	Yield (%)	M_n[b]	M_w[b]	M_w/M_n
A	0	0	95	32,200	69,600	2.2
B	15	8	84	15,500	34,200	2.2
C	23	18	87	13,400	30,600	2.3
D	32	30	85	11,800	25,500	2.1
E	41	41	82	9200	16,600	1.8

[a] Calculated from ^{1}H NMR measurement.
[b] Determined by GPC measurement.

GPC curves of protected copolymers are shown in Fig. 1. The molecular distribution of the copolymer became relatively narrow with increasing MA content in the feeding dose. The molecular weights of the copolymers obtained with different feeding doses were determined by GPC as shown in Table 1. The molecular weight of the copolymers decreased as the MA content increased and was lower than that of the PLLA homopolymer, which was prepared under the same conditions. This was due to transesterification, which commonly occurs in ring-opening polymerization. The pendant benzylester groups of the copolymers reacted with the monomers; they terminated the polymerization and reduced the molecular weight of copolymers. The composition of poly(L-lactide-*co*-*RS*-β-benzyl malate) was determined by ^{1}H NMR measurements. All the proton signals displayed by the copolymer are assigned in Fig. 2. By integration of band f and c, the L-LA and MA contents in the copolymers were calculated and are listed in Table 1. It can be seen that the MA content in the copolymers changed regularly with the feeding dose, i.e. the higher the MA level in the feeding dose, the more MA units that were incorporated in the copolymers. However, it was surprising that the copolymer compositions were identical to the feeding doses if the MA content was relatively high, such as above 30 mol%. When the MA contents in the feeding dose



Scheme 1. Synthetic route of poly(L-lactide-*co*-*RS*-β-malic acid).

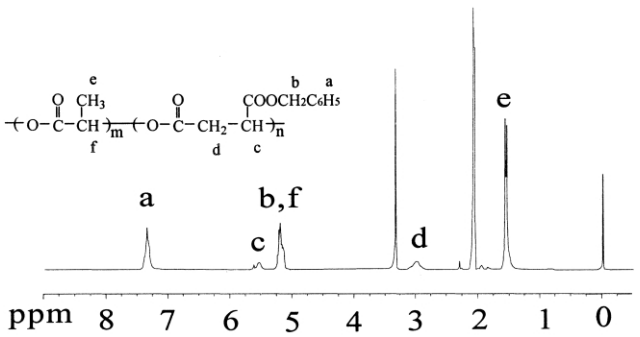


Fig. 2. A typical ^{1}H NMR spectrum of poly(L-lactide-*co*-*RS*-β-benzyl malate). The peaks at 2.07 and 3.33 ppm are the protons of water and acetone.

were lowered, e.g. 23 and 15 mol%, the compositions of the copolymers deviated much from the feeding dose as only part of the MA entered the polymeric chains. This was attributed to the different activities of the two monomers. The reaction activity of L-LA was higher than that of MA.

3.2. Deprotection of copolymers

Poly(L-lactide-*co*-*RS*-β-malic acid) was obtained by deprotection (hydrogenolysis) of poly(L-lactide-*co*-*RS*-β-benzyl malate). The deprotection was confirmed with ^{1}H NMR and FTIR as shown in Figs. 3 and 4, respectively. The two proton peaks due to the benzyl groups disappeared after hydrogenolysis, while the other proton peaks were not changed (Fig. 3). In the FTIR spectra, the δ CH vibration of the benzyl group at 754 and 700 cm^{-1} disappeared and the vibration of OH in the carboxyl group at 3449 cm^{-1} was strengthened greatly after hydrogenolysis. These results clearly showed that the benzyl groups had been removed. GPC analyses of the de-protected copolymers are shown in Table 2. The molecular weight of the de-protected copolymer was larger than that of protected copolymer. This was attributed to the intermolecular force increase resulting from the formation of hydrogen bonds among the carboxyl groups. The same phenomenon was also observed in other copolymers with hydrophilic pendant groups [21].

The variation of the water contact angles of copolymer

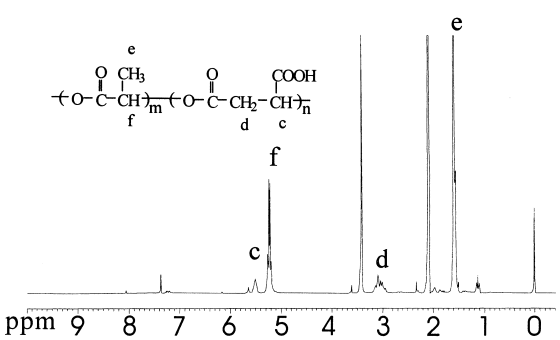


Fig. 3. ^{1}H NMR spectrum of poly(L-lactide-*co*-*RS*-β-malic acid). The peaks at 2.07 and 3.40 ppm are the protons of water and acetone.

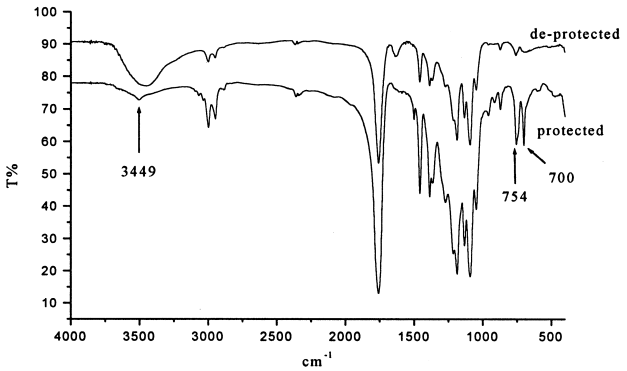


Fig. 4. FTIR spectra of poly(L-lactide-*co*-*RS*-β-benzyl malate) and poly(L-lactide-*co*-*RS*-β-malic acid).

films are shown in Table 3. It can be seen that the water contact angle decreased greatly due to the introduction of carboxyl groups into copolymers. The hydrophilicity of the de-protected copolymer was higher than that of the PLLA homopolymer, and it increased with increasing malic acid content in the copolymers.

3.3. Thermal properties of protected and de-protected copolymers

The thermal properties of poly(L-lactide-*co*-*RS*-β-benzyl malate) were determined by DSC analysis as shown in Fig. 5. It is clear that the crystallinity of the copolymers decreased as the MA content increased. The morphology of the copolymers changed from crystalline to amorphous as the MA content increased from 8 to 41 mol%. The crystallization behaviors were very different, in sample B, both crystallization and crystal-melting peaks are obvious, while in sample C and D, only a crystal-melting peak can be observed. In sample E, no crystallization or crystal-melting peaks are present. The crystal-melting temperature (T_m) and glass transition temperature (T_g) of the copolymers also decreased as the MA content increased. Poly(*RS*-β-benzyl

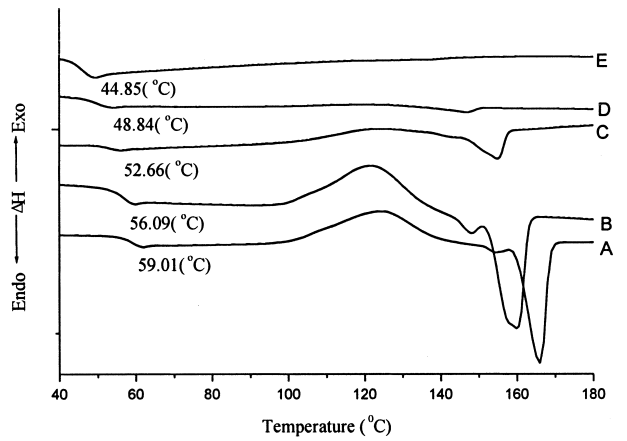


Fig. 5. DSC spectra of PLLA and poly(L-lactide-*co*-*RS*-β-benzyl malate). Composition of L-LA/MA was (A) 100/0, (B) 92/8, (C) 82/18, (D) 70/30, (E) 59/41.

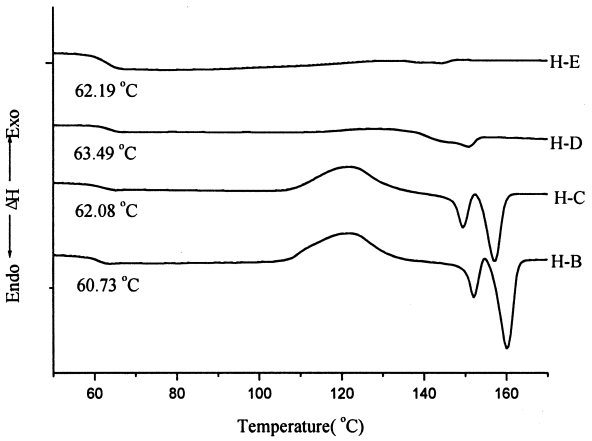


Fig. 6. DSC spectra of poly(L-lactide-*co*-*RS*-β-malic acid). Composition of L-LA/MA was (H-B) 92/8, (H-C) 82/18, (H-D) 70/30, (H-E) 59/41.

malate) is an amorphous polymer with a low glass transition temperature (T_g). The random MA component in the copolymers destroyed the regularity of the PLLA chain. This led to a decrease in the crystallinity, the crystal-melting temperature (T_m) and the glass transition temperature (T_g).

On the other hand, the T_g of all the de-protected copolymers was higher than that of the corresponding protected copolymers (Fig. 6). This was attributed to the formation of intermolecular hydrogen bonds between the carboxyl groups. Hydrogen bonds also hampered the movement of the polymeric chains and limited the chains to certain domains, thus, imperfect crystal were formed. The existence of imperfect crystal domains and the crystals formed during the DSC scanning processes led to two endothermic peaks in sample H-B and H-C. The T_m of the de-protected copolymers was sharper and higher than that of the protected copolymers because the weight percentage of L-LA units was increased in the copolymers upon the benzyl groups' removal [21]. Only one glass transition appeared in the DSC spectra of both the protected and de-protected copolymers further indicated that the copolymers were true copolymers and not just a mixture of PLLA and PMA homopolymer.

4. Conclusion

Novel copolymers with functional pendant groups were obtained by hydrogenolysizing copolymers of L-LA and MA. The protected poly(L-lactide-*co*-*RS*-β-benzyl malate) had lower T_g, while the de-protected poly(L-lactide-*co*-*RS*-β-malic acid) exhibited higher T_g than that of PLLA. The molecular weight of the copolymers decreased as the MA content increased, and the morphology of copolymers changed from crystalline to amorphous. The hydrophilicity was increased by the introduction of malic acid units into the de-protected copolymers. The copolymer pendent carboxyl groups should be useful to immobilize bioactive molecules such as amino acids, peptides and proteins on its surface. It has potential as a cell-compatible material in the biomedical field.

Table 2
Molecular weight of poly(L-lactide-*co*-*RS*-β-malic acid)

De-protected sample	De-protected copolymer[a]		
	M_n	M_w	M_w/M_n
H-B	20,600	46,800	2.3
H-C	31,300	63,800	2.0
H-D	13,500	27,100	2.2
H-E	_[b]	_[b]	_[b]

[a] Determined by GPC measurement.
[b] The copolymer could not dissolve in chloroform.

Table 3
Water contact angle of poly(L-lactide-*co*-*RS*-β-benzyl malate) and poly(L-lactide-*co*-*RS*-β-malic acid)

Sample	Water contact angle (degree)	
	Protected copolymer	De-protected copolymer
A	78	78
B	79	60
C	76	55
D	76	47
E	77	23

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