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RESEARCH ARTICLE

Synthesis of depsipeptides from *L*-amino acids and lactones

Hongfei CAO[1], Yakai FENG (✉)[1,2], Heyun WANG[1], Li ZHANG[1], Musammir KHAN[1], Jintang GUO[1,2]

1 School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
2 Tianjin University-Helmholtz-Zentrum Geesthacht, Joint Laboratory for Biomaterials and Regenerative Medicine, Tianjin 300072, China; Teltow 14513, Germany

Abstract By using the corresponding *L*-amino acid sodium as initiator, ε-caprolactone-depsipeptides CL-Ala and CL-Leu were prepared by the reactions of ε-caprolactone (CL) with *L*-alanine and *L*-leucine, respectively, and *p*-dioxanone-depsipeptide (PDO-Leu) was prepared by the reaction of *p*-dioxanone (PDO) with *L*-leucine. Two poly(ε-caprolactone) oligomers (PCL-Ala and PCL-Leu) of different molecular weights with depsipeptide unit were synthesized by controlling the feed ratio of *L*-amino acid sodium and CL. The presence of the depsipeptide structure in these obtained products was confirmed by ^{1}H NMR spectra and the molecular weight of the poly(ε-caprolactone) oligomers was measured by gel permeation chromatography (GPC). These products contain a hydroxyl group and a carboxyl group in one molecule, which means they could act as bifunctional monomers for further polymerization to prepare high molecular weight polymers. By this way, the depsipeptide unit could be introduced into the polymers and the biodegradation rates of the novel polymers could be well controlled in vivo by the tailored molecular structures.

Keywords ε-caprolactone, *p*-dioxanone, *L*-alanine, *L*-leucine, depsipeptide

1 Introduction

During the past decades, much attention has been paid to the biodegradable polymers due to the environmental significance [1] and their important biomedical and pharmaceutical applications, particularly in drug delivery matrices, wound healing and tissue reconstruction [2,3]. Owning to the lack of toxicity, attractive crystallinity, excellent thermal properties, biocompatibility, biodegradability, and miscibility with other polymers, aliphatic polyesters become a series of well-known biodegradable synthetic polymers and several aliphatic polyesters form an important class of biomaterial with excellent biocompatibility [4–6], such as poly(*L*-lactide) (PLA) [7,8], poly(ε-caprolactone) (PCL) [9,10], poly(*p*-dioxanone) (PPDO) [11] and their copolymers [12]. Zhang et al. [13] prepared poly(*L*-lactide)-poly(ethylene glycol)-poly(*L*-lactide) hydrogels as novel thymopentin release systems. The results indicated that higher copolymer concentration led to slower release rate. Li et al. [14] had synthesized core-sheath nanofibers composed of PCL and silk fibroin (SF) blends via emulsion electrospinning and evaluated the potential of fabricated PCL/SF composite nanofibers as scaffold in vitro. The results confirmed that fabricated PCL/SF scaffolds improved cell attachment and proliferation. Liu et al. [15] had prepared copolymer of chitosan-*g*-poly(*p*-dioxanone) as ibuprofen carrier, and the release rate of ibuprofen decreased compared with that of pure chitosan carrier. However, the mentioned polymers (PLA, PCL and PPDO) which were commercially available had degradation time of either a couple of months or over a year by simple hydrolysis in vivo [16]. PCL was almost stable against hydrolytic attack for about nine months in hydrolytic degradation experiments, and the weight loss of the PPDO samples was only 25% in 10 weeks [17,18]. The difficulty of the degradation has significantly restricted the practical application like drug delivery systems in medical field.

Because depsipeptide unit has the potential of biodegradability due to the incorporation of *L*-amino acids, which can be targeted for cleaving by enzymes, such as proteases [19,20], and the degradation products *L*-amino acids are nontoxic and can be metabolized properly by living tissues [21,22]. Many efforts had developed to introduce *L*-amino acids into polymer backbone for the depsipeptide [23], such as the synthesis of poly(depsipeptide-co-lactide)-*g*-poly(ethylene glycol) copolymers [24], poly(depsipeptides)-*b*-poly(ethylene glycol)-*b*-poly(depsipeptides) [25] and poly(depsipeptides) [26–28]. Ohya et al.

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E-mail: yakaifeng@tju.edu.cn

[29] had synthesized poly[(glutamic acid-aspartic)-co-lactic acid] with depsipeptide units. In cell culture, the copolymer films exhibited higher degradation rate which was related to the depsipeptide units and changed with varying depsipeptide content. Ouchi et al. [30] had found that the solubility, thermal transition and degradation behavior of the modified PLA could be varied by changing the mole fraction of the depsipeptide content in the copolymer. Ohya et al. [31] had synthesized amphiphilic ABA-type triblock copolymers. The depsipeptide unit content in the molecular architecture affected the degradation rates of the polymer films significantly. We inferred possible and promising approaches to overcome the poor degradability should be the introduction of depsipeptide unit into polymer backbone.

The major studies concerned the methods for the introduction of depsipeptide unit into the PLA [32]. Li et al. [33] had synthesized the polymorpholine-2,5-dione-block-polylactide via ring-opening polymerization of morpholine-2,5-dione and lactide. Xie et al. [34] had prepared poly{(lactic acid)-co-[(glycolic acid)-alt-(*L*-glutamic acid)]}-block-poly(ethylene glycol)-block-poly{(lactic acid)-co-[(glycolic acid)-alt-(*l*-glutamic acid)]} via the ring-opening copolymerization of *L*-lactide and (3s)-benzoxylcarbonylethyl-morpholine-2,5-dione in the presence of dihydroxyl poly(ethylene glycol). However, to our best knowledge, there are few reports in the literature about the introduction of depsipeptide unit into PCL and PPDO.

To introduce depsipeptide content into PCL and PPDO, the key issue is the preparation of the monomers, intermediate or oligomers. In this article, ε-caprolactone-depsipeptide with *L*-alanine (CL-Ala), ε-caprolactone-depsipeptide with *L*-leucine (CL-Leu), *p*-dioxanone-depsipeptide with *L*-leucine (PDO-Leu) and two PCL oligomers (PCL-Ala and PCL-Leu) with depsipeptide unit were synthesized by using the corresponding amino acid sodium as the initiator. In the obtained products, the functional end groups of hydroxyl and carboxyl are available for the further polymerization. Then, the depsipeptide unit will be incorporated into the PCL and PPDO backbones, and the biodegradation rates of novel synthesized copolymers will be potentially controlled for specific application.

2 Materials and methods

2.1 Materials

ε-Caprolactone (CL, 99%, analytical grade) and *p*-dioxanone (PDO, 99%, analytical grade) were purchased from Aladdin (Shanghai, China). *L*-alanine (food grade) and *L*-leucine (food grade) were from Guangfu Chemical Co. (Tianjin, China). CL and PDO were dried over CaH_2 for 2 days and then distilled under reduced pressure prior to use. Stannous octanoate [$Sn(Oct)_2$] (95%) was purchased from Sigma. Hydrochloric acid (HCl), toluene, 1,4-dioxane and other reagents were of analytical grade.

2.2 Preparation of *L*-amino acid sodium

Alcohol (40 mL) was taken into a dry beaker, and then Na (0.727 g, 31.6 mmol) was added into it. The reaction was kept at room temperature. Until the sodium pieces disappeared completely, *L*-alanine (2.809 g, 31.6 mmol) was added into the mixture. The mixture was then stirred for several minutes until the *L*-alanine powder disappeared. At the end of the reaction, the alcohol was removed by evaporation, and a white solid was obtained. The *L*-alanine sodium was dried in vacuum oven to constant weight (97% yield) for further experiment. The *L*-leucine sodium (97% yield) was prepared by the same process as *L*-alanine sodium.

2.3 Preparation of CL-Leu

CL (12.262 g, 108 mmol), *L*-leucine sodium (4.230 g, 28 mmol) were mixed in a round bottom flask equipped with a magnetic stirrer. To the resultant mixture, anhydrous 1,4-dioxane (15 mL) was added. The reaction was allowed to proceed for 4 h in a water bath at 60°C under N_2 atmosphere. Following the reaction, a white solid appeared in the solution. After predetermined reaction time, the 1,4-dioxane was removed from the flask, and the solid was washed three times with chloroform. Then the solid was dissolved in distilled water and the pH of the solution was adjusted to 2 with HCl (0.1 mol/L). After the water was removed by evaporation, the residue was redissolved in acetone, and the precipitates were filtered off. The filtrate was evaporated under reduced pressure to give CL-Leu which was dried under vacuum to constant weight.

2.4 Preparation of CL-Ala

CL (2.485 g, 22 mmol), *L*-alanine sodium (2.481 g, 22 mmol) were introduced into a dried flask equipped with a magnetic stirrer and the reaction was carried out at 72°C for 4 h under N_2 atmosphere. After the flask was cooled down to room temperature, a white solid was appeared. The solid was dissolved in distilled water, and then the pH of the solution was adjusted to 2 with HCl (0.1 mol/L). To remove the unreacted *L*-alanine, this solution was extracted three times with ethyl acetate. The extracts were washed with water and then dried over anhydrous sodium sulfate. Filtration to remove sodium sulfate and evaporation under reduced pressure to give CL-Ala which was dried under vacuum to constant weight.

2.5 Preparation of PDO-Leu

PDO (1.736 g, 17.0 mmol), *L*-leucine sodium (3.539 g,

23.1 mmol) and anhydrous toluene (15 mL) were transferred into a dried flask in sequence. The reaction was performed for 4 h at 60°C under N_2 atmosphere with magnetic stirring. Then the resultant mixture was filtered and the white precipitates were washed three times with chloroform and further treated according to the same procedure for CL-Leu.

2.6 Preparation of PCL-Ala and PCL-Leu

Predetermined amount of *L*-amino acid sodium, and CL: $Sn(Oct)_2$ (mole ratio = 1000 : 1) were introduced into a pre-dried flask in sequence. The reaction was performed at 140°C for 7 h under N_2 atmosphere in an oil bath. After the polymerization almost completed, the reaction mixture turned into a white solid after cooled down to room temperature. The white solid was dissolved in chloroform and the solution was acidified with the same volume of HCl. With removal of the HCl solution, the organic phase was added dropwise into a mixure of diethyl ether and petroleum ether ($V : V = 1 : 1$) under vigorous stirring. The precipitates were filtrered and dried at room temperature in a vacuum. PCL-Ala and PCL-Leu with different molecular weights were obtained by controlling the mole ratio of CL and *L*-amino acid sodium as shown in Table 1.

2.7 Measurement methods

The CL-Ala, CL-Leu, PCL-Ala and PCL-Leu samples were dissolved in chloroform-*d*, and PDO-Leu was dissolved in DMSO-d_6 for ^{1}H NMR analysis. NMR data was typically collected at 25°C in ppm relatively to solvent signal using ECA-500 spectrometers at 400 MHz.

The molecular weight of the resulting PCL oligomers was determined by gel permeation chromatography (GPC) using a Kontron HPLC-420 instrument. The measurements were performed using tetrahydrofuran as eluent at a flow rate of 1.0 mL/min at 40ºC and a series of polystyrene as standards.

3 Results and discussion

3.1 Synthesis of CL-Ala, CL-Leu and PDO-Leu

To impart the depsipeptide unit to polymers, the preparation of monomers, intermediate or oligomers is critical. Several papers introduced depsipeptide to PCL and PPDO via the copolymerization of *N*-carboxyanhydrides and amino terminated lactones, such as the syntheses of poly (caprolactone)-*b*-poly(benzyl-*L*-glutamic acid), poly (asparagine-*g*-caprolactone), poly(glycine)-*b*-poly(caprolactone) and poly(alanine)-*b*-poly(caprolactone) [35–37]. However, the copolymerization process is very complicated [38] and costly. The *L*-amino acid *N*-carboxyanhydrides are difficult to prepare and store because of the moisture sensitivity. These shortcomings restrict the application of *N*-carboxyanhydrides for the synthesis of copolymers with depsipeptide unit. An alternative way through copolymerization of morpholine-2,5-dione derivatives and lactones [39] also comprises some drawbacks. First, there are limited available routes and low yields for synthesizing morpholine-2,5-dione derivatives. Second, the severe reaction conditions may cause undesirable side reactions and unexpected chemical units in the polymer backbone [21]. To overcome these drawbacks, we used a simple synthetic route for three novel intermediates as shown in Fig. 1. By the attack of the primary amino group

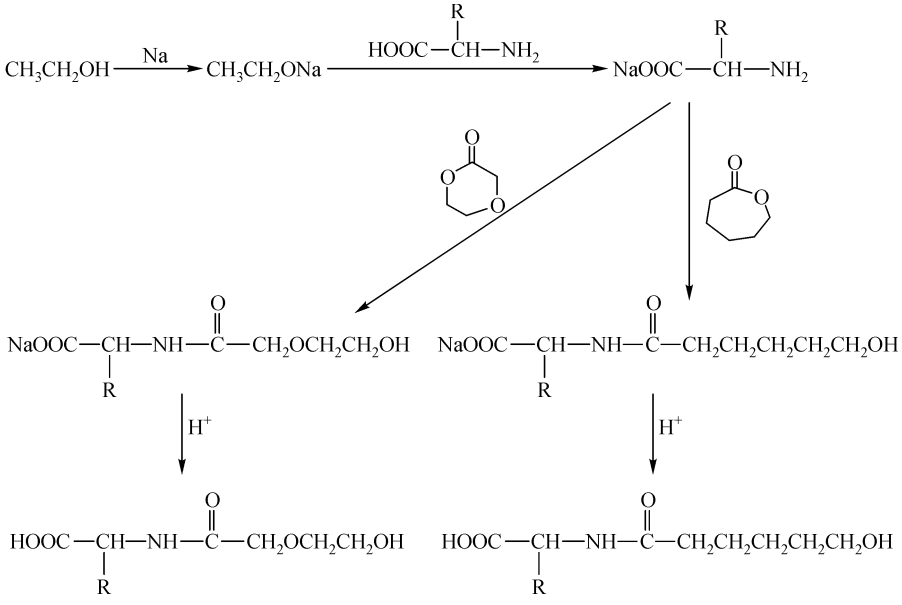

Fig. 1 Synthetic routes for the depsipeptides from lactones

of *L*-amino acid sodium, the ring-opening of CL and PDO proceeded and the ester bond was transformed into amide bond. The CL-Ala, CL-Leu and PDO-Leu containing the end groups of hydroxyl and carboxyl were synthesized under mild conditions. The chemical structures of these compounds were confirmed by means of ^{1}H NMR spectra.

3.2 Chemical structure identification of CL-Ala and CL-Leu

The chemical structure of CL-Ala was shown in Fig. 2(a). The signal marked 'b' at chemical shift (δ) 4.67 ppm was ascribed to the –CH–N proton of the *L*-alanine residue. The peak marked 'c' at δ 6.70 ppm corresponded to the O = C–NH– proton. These two signals confirmed the formation of depsipeptide unit from the amino and ester group in CL-Ala. The peaks marked 'i', 'd', 'e + h', 'f' and 'j' were ascribed to ε-caprolactone segment protons. The peak marked 'm' at δ 4.10 ppm confirmed the presence of a hydroxyl group at one end of CL-Ala. The ^{1}H NMR spectrum confirmed clearly the structure of the CL-Ala with depsipeptide unit.

In ^{1}H NMR spectrum of the CL-Leu (Fig. 2(b)), the typical signals of ε-caprolactone segment and *L*-alanine unit protons were detected. The signals at δ 0.91 ppm and 4.56 ppm were ascribed to the $–CH_3$ and –CH–N protons of the *L*-leucine residue, respectively. The peak at δ 6.95 ppm corresponded to the O = C–NH– proton. The three signals confirmed the formation of depsipeptide unit from the amino acid and ester.

3.3 Chemical structure identification of PDO-Leu

The ^{1}H NMR spectrum of PDO-Leu was shown in Fig. 3. The typical signals of the *p*-dioxanone segment and *L*-leucine unit protons were detected in the ^{1}H NMR spectrum. The peak marked 'e' at δ 7.94 ppm corresponded to the O = C–NH– proton. The two signals marked 'f' at δ 3.87 ppm and marked 'a' at δ 0.87 ppm were ascribed to the O = C–CH_2–O– protons of the *p*-dioxanone segment and the $–CH_3$ protons of the *L*-leucine unit, respectively. The signal marked 'i' at δ 4.02 ppm indicated the presence of a hydroxyl group (–OH) at one end of PDO-Leu. The various proton signals confirmed that *L*-leucine sodium could initate the ring-opening of the PDO and form the structure of the depsipeptide. Comparison of the integrations of the protons peaks marked 'a' and 'f' suggested that the mole ratio of the *L*-leucine unit and the *p*-dioxanone segment in PDO-Leu was 1 : 1. The hydroxyl and carboxyl end groups made the PDO-Leu available for alternating copolymer.

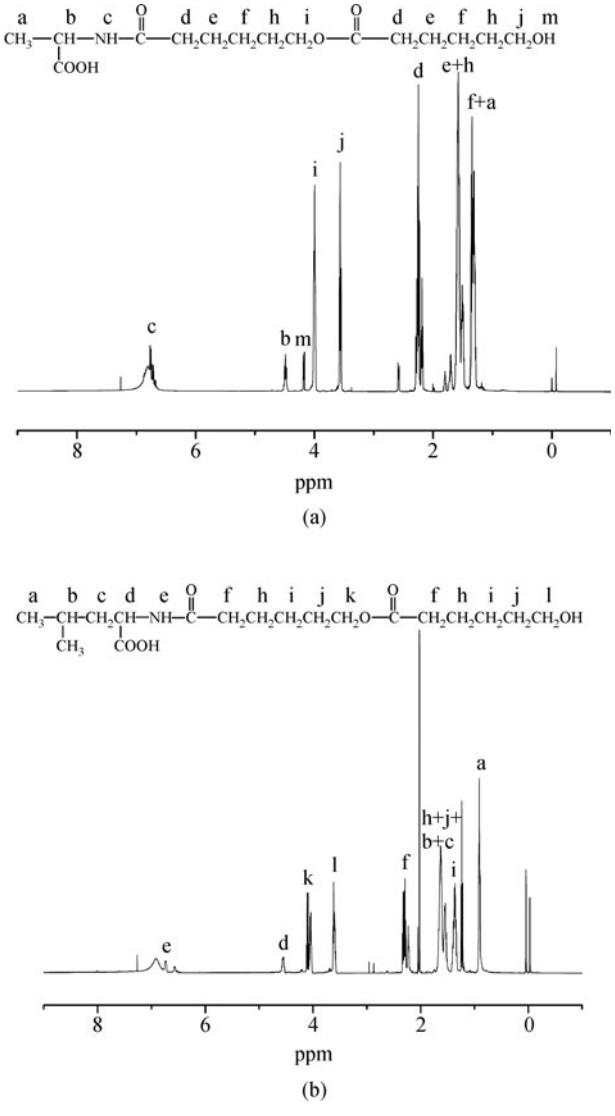

Fig. 2 ^{1}H NMR spectrum of CL-Ala (a) and CL-Leu (b) in $CDCl_3$ at 25°C

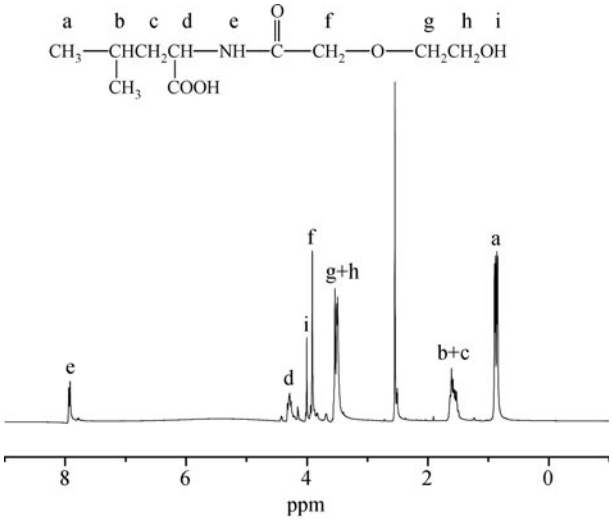

Fig. 3 ^{1}H NMR spectrum of PDO-Leu in DMSO-d_6 at 25°C

Table 1 The molecular weight of the PCL-Ala and PCL-Leu

Sample ID	CL: amino acid sodium (mol: mol)	Temperature/°C	Reaction time/h	M_n
PCL-Ala_1	20	140	7	2172
PCL-Ala_2	40	140	7	4421
PCL-Ala_3	85	140	7	9779
PCL-Leu_1	20	140	7	2195
PCL-Leu_2	40	140	7	4197
PCL-Leu_3	85	140	7	9592

3.4 Synthesis of PCL-Ala and PCL-Leu

In this paper, different molecular weights of PCL-Ala and PCL-Leu were synthesized by the initiation of *L*-alanine sodium and L-leucine sodium, respectively. The molecular weights of the PCL oligomers were measured by ^{1}H NMR and the results were showed in Table 1. The M_w/M_n of PCL-ala_1 (M_n = 2172) and PCL-leu_1 (M_n = 2195) were 1.33 and 1.48 by GPC, respectively.

The chemical structures of PCL-Ala and PCL-Leu were confirmed by ^{1}H NMR spectra and the ^{1}H NMR spectrum of PCL-Ala (M_n = 2172) was shown in Fig. 4. The signal at δ 6.40 ppm was corresponded to the O = C–NH– proton, and the signal marked 'b' at δ 4.75 ppm originated from the –CH–N proton of the *L*-alanine residue. The peak marked 'm' at δ 4.50 ppm indicated the presence of a hydroxyl group at one end of PCL-ala. The typical peaks of the PCL segment were marked 'i', 'j', 'd' and 'e + h', respectively. The various proton signals confirmed the presence of the depsipeptide unit in the PCL-Ala. Such results implied that poly(ε-caprolactone) oligomers of different molecular weights with the depsipeptide unit could be obtained by controlling the feed molar ratio of *L*-amino acid sodium and CL.

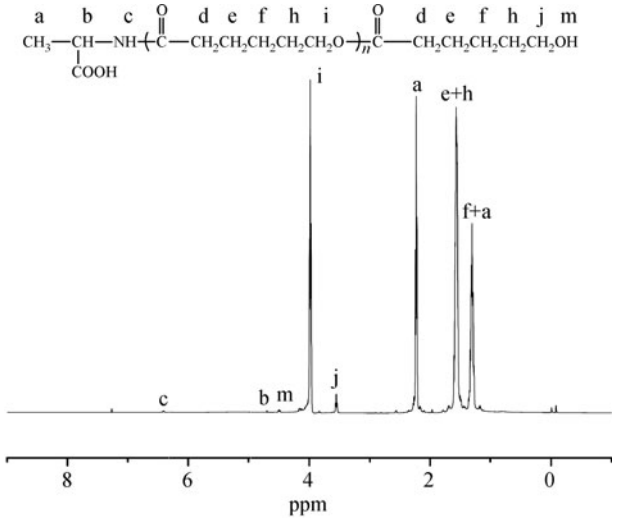

Fig. 4 ^{1}H NMR spectrum of PCL-Ala in $CDCl_3$ at 25°C (M_n = 2172)

4 Conclusions

In this study, a simple route was provided for the synthesis of CL-Ala, CL-Leu, PDO-Leu and PCL oligomers of different molecular weights with the depsipeptide unit. These depsipeptides were obtained from the ring-opening of CL or PDO by using the corresponding *L*-amino acid sodium as initiator. The chemical structures of these products were identified by ^{1}H NMR spectra and the molecular weight of PCL oligomers was measured by GPC. Our results indicated the presence of depsipeptide unit in CL-Ala, CL-Leu and PCL oligomers. These products contained a hydroxyl group and a carboxyl group, which can be used as monomers for further polymerization to prepare high molecular weight copolymers with the depsipeptide unit in their backbones. Thus the biodegradation rate of these biomaterials can potentially be controlled for future biomedical application.

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