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ORIGINAL PAPER

Synthesis and characterization of biodegradable poly(ε-caprolactone)/poly(γ-benzyl L-glutamate) block copolymer

Qiuhua Wu · Linyao Zhou · Dan Zhang · Ximing Song · Guolin Zhang

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Abstract A biodegradable poly(ε-caprolactone)/poly(γ-benzyl L-glutamate) (PCL-*b*-PBLG) block copolymer was synthesized by ring-opening polymerization of *N*-carboxy-γ-benzyl L-glutamate anhydride (BLG-NCA) with amine-terminated poly (ε-caprolactone) (PCL-NH_2) as a macroinitiator. The PCL-NH_2 was prepared by deprotection of a PCL-CH_2CH_2NHBoc, which was obtained by ring-opening polymerization of ε-caprolactone (ε-CL) initiated by Boc-aminoethanol ($HOCH_2CH_2NHBoc$) using stannous octanoate as catalyst under microwave irradiation. The structures of the block copolymers were determined by IR, ^1H NMR, and GPC measurements. The results prove that BLG-NCA can be initiated by PCL-NH_2 to produce PCL-*b*-PBLG block copolymers.

Keywords Biodegradable block copolymers · Poly(ε-caprolactone) · Poly(γ-benzyl L-glutamate) · Microwave-assisted synthesis · Ring-opening polymerization

Abbreviations

PCL-*b*-PBLG	Poly(ε-caprolactone)/poly(γ-benzyl L-glutamate)
BLG-NCA	*N*-carboxy-γ-benzyl L-glutamate anhydride
PCL-NH_2	Amine-terminated poly(ε-caprolactone)
ε-CL	ε-Caprolactone
$HOCH_2CH_2NHBoc$	Boc-aminoethanol
PAA	Poly(α-amino acid)s

Q. Wu · L. Zhou · X. Song · G. Zhang (✉)
Liaoning Provincial Key Laboratory for Green Synthesis and Preparative Chemistry of Advanced Materials, College of Chemistry, Liaoning University, Shenyang 110036, People's Republic of China
e-mail: glzhang@lnu.edu.cn

D. Zhang
Fushun New and High-Tech Industry Development Zone Management Committee, Fushun 113004, People's Republic of China

$Sn(Oct)_2$	Stannous octoate
Boc	*t*-Butoxycarbonyl
$H_2NCH_2CH_2OH$	2-Aminoethanol
THF	Tetrahydrofuran

Introduction

Recently, biodegradable polymers have attracted much attention because biodegradability is extremely significant properties for some applications of biomedical polymers. They are widely used in numerous medical and pharmaceutical applications because of their low immunogenicity and good biocompatibility [1–3]. Polyesters, such as poly(lactic acid) and poly(ε-caprolactone) are biodegradable polymers. They are used as resorbable suture materials and drug controlled release systems [4–6].

Poly(α-amino acid)s (PAAs) are well known as very important synthetic biodegradable materials. They can be synthesized by means of ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCA) initiated by primary amine and their biodegradation in mammalian tissues can be well controlled by their structure [7]. Due to their polypeptide backbone, they have the potential to be degraded in biological environments. Moreover, since they have low immunogenicity, good biocompatibility, and excellent mechanical properties, they may be widely used in pharmaceutical and other medical applications [8–10]. The main potential medical applications for synthetic α-amino acid-based polymers are controlled release of drug implants and proteins, scaffolds for tissue engineering and medical devices. There are many reports about copolymers of α-amino acids and polyesters. Chen [11] had synthesized poly(α-amino acid)/poly(ε-caprolactone) block copolymer by using amino calcium 4-nitrobenzoxide as initiator. Kricheldorf [12] had synthesized poly(α-amino acid)/poly(ε-caprolactone)/poly-(α-amino acid) block copolymer by using 2,2-dibutyl-2-stanna-1,3-dioxepane as initiator. Höcker [7] had synthesized poly(α-amino acid)/poly(L-lactide) block copolymer by using diethylzinc catalyst. Xiong [13] had synthesized poly [(glycolic acid)-alt-(L-glutamic acid)] and poly {(lactic acid)-co-[(glycolic acid)-alt-(L-glutamic acid)]} by using stannous octanoate as catalyst. Guillaume [14] had synthesized polypeptide/poly(ε-caprolactone)/polypeptide block copolymer by using La $(OiPr)_3$ as catalyst.

As an environmentally benign process, microwave irradiation has been developed into a highly useful technique and an effective alternative energy source for chemical reactions and processes [15]. Microwave irradiation has been shown not only to reduce reaction times, but also to provide selective and higher yields of the desired products, as compared to traditional heating methods [16]. Over the last decade, microwave heating has been widely applied in polymer synthesis, such as polycondensation, free and controlled radical polymerization, and ring-opening polymerization [17, 18].

In this article, we report a novel synthesis of biodegradable poly(ε-caprolactone)/poly(γ-benzyl L-glutamate) (PCL-*b*-PBLG) block copolymer by ring-opening polymerization of *N*-carboxy-γ-benzyl L-glutamate anhydride (BLG-NCA) initiated by amine-terminated poly(ε-caprolactone) (PCL-NH_2), which was obtained by ring-opening polymerization of ε-caprolactone (ε-CL) initiated by Boc-aminoethanol ($HOCH_2CH_2NHBoc$) using stannous octanoate as catalyst under microwave irradiation. The structures of the block copolymers were determined by IR, ^1H NMR, and GPC measurements.

Experimental

Materials

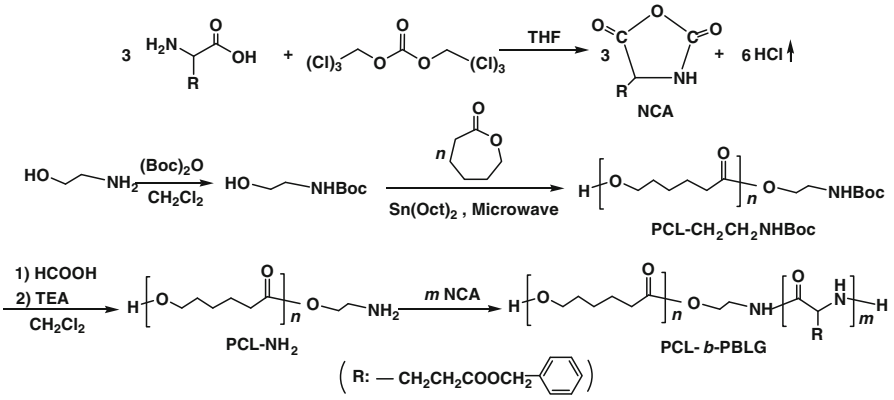
The ε-CL was purchased from Alfa Chemical Reagent Co., Ltd. L-Glutamic acid (biochemical grade), Stannous octoate ($Sn(Oct)_2$), Boc_2O (Boc: *t*-butoxycarbonyl, analytical grade), 2-aminoethanol ($H_2NCH_2CH_2OH$, analytical grade), tetrahydrofuran (THF, analytical grade), and dichloromethane (CH_2Cl_2, analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), dichloromethane and tetrahydrofuran were dried and distilled before use. Triphosgene (chemical grade) was obtained from Haining Zhonglian Chemical Reagent Co., Ltd. (Zhejiang, China), used without any treatment. Silica gel for column chromatography (ZCX-II) was purchased from Qingdao Haiyang Chemical Co., Ltd. (Shandong, China). Other chemicals are all analytical reagents made in China and used without further purification.

Preparation of γ-benzyl L-glutamate and BLG-NCA

γ-Benzyl L-glutamate was prepared from L-glutamic acid and benzyl alcohol (Scheme 1), melting point 172–174 °C [19]. Anal. calcd. for $C_{12}H_{15}NO_4$ (%): C: 60.75, H: 6.37, N: 5.90. Found (%): C: 60.48, H: 6.62, N: 5.97. BLG-NCA was prepared by the reaction of γ-benzyl L-glutamate with triphosgene in dried THF at 50 °C according to a literature procedure, melting point 96–97 °C [20]. Anal. calcd. for $C_{13}H_{13}NO_5$ (%): C: 59.31, H: 4.98, N: 5.32. Found (%): C: 59.54, H: 4.87, N: 5.26. ^1H NMR ($CDCl_3$, δppm): 2.08, 2.29 ($–CH_2OOCCH_2\mathit{CH_2}–$, 2H), 2.57 ($–CH_2OOC\mathit{CH_2}CH_2–$, 2H), 4.38 (–*CH*, 1H), 5.13 ($–\mathit{CH_2}OOCCH_2CH_2–$, 2H), 6.52 (N*H*, 1H), 7.37(Ar–*H*, 5H). The reaction route is shown in Scheme 1.

Preparation of PCL-NH_2 [21]

0.61 g (10 mmol) of 2-aminoethanol was dissolved in 20 mL $CHCl_3$ in a flask equipped with a magnetic stirrer, and 2.4 g (11 mmol) of Boc_2O in $CHCl_3$ (20 mL) was added. After stirring for 30 min, the concentrated solution was diluted with 20 mL 5% potassium hydrogen sulfate and extracted with ethyl acetate and dried over sodium sulfate. The purification of the compound was carried out by column



Scheme 1 The route for the synthesis of PCL-*b*-PBLG block copolymer

chromatography on silica gel using chloroform and methanol (v/v = 70:1) as eluent to give $HOCH_2CH_2NHBoc$. Yield: 85.7%.

The polymerization was carried out in a Nanjing Jiequan microwave oven, with a working frequency of 2.45 GHz and a maximum output power of 850 W. Typically, 3.75 g of ε-CL, 0.018 g of $Sn(Oct)_2$, and 0.11 g of $HOCH_2CH_2NHBoc$ were added to a 50 mL round bottomed flask equipped with a nitrogen gas inlet and a condenser, and the solution was subjected to microwave irradiation under stirring for 30 min (500 W). The resulting mixture was immersed into an ice water bath and the precipitate was filtered. The crude product of PCL-CH_2CH_2NHBoc was dissolved in 20 mL of dichloromethane and precipitated in 300 mL methanol. The purified product was dried under vacuum at 25 °C.

The PCL-NH_2 was prepared by the removal of the Boc group from the PCL-CH_2CH_2NHBoc. Typically, PCL-CH_2CH_2NHBoc was treated with 10 mL of formic acid at room temperature for 12 h under stirring. Then the solution was poured into a 300 mL of methanol to obtain the precipitate. The precipitate was dried under vacuum at 25 °C, and then dissolved in 20 mL of CH_2Cl_2. 10 mL of triethylamine was added and the mixture was kept at room temperature for 12 h under stirring. The resulting solution was poured to 200 mL of methanol and kept at 2 °C for 12 h. The precipitate was filtered off and dried in vacuum at 25 °C to give the desired PCL-NH_2. The reaction route is shown in Scheme 1.

Synthesis of PCL-*b*-PBLG block copolymer

Certain amounts of PCL-NH_2 and BLG-NCA were dissolved together in 40 mL anhydrous CH_2Cl_2 and kept at 30 °C for 72 h under stirring. The mixture was poured to 200 mL of methanol to give white precipitate. The precipitate was filtered off and dried in vacuum at 25 °C for 20 h to give the desired PCL-*b*-PBLG block copolymers. The reaction route is shown in Scheme 1. Different molar ratios of the feeding BLG-NCA to PCL-NH_2 resulted in the corresponding copolymers with various compositions as listed in Table 1.

Table 1 Related data on PCL-*b*-PBLG block copolymers

Samples	PCL-NH_2/BLG-NCA[a]	W_{PCL}/W^{b}_{PBLG}	Yield (%)	M^{b}_{n}	M^{c}_{n}	M_w/M_n
PCL-NH_2	–	–	80.3	4560	15375	1.55
PCL-*b*-PBLG30	1/30	87/13	84.6	5217	16710	1.68
PCL-*b*-PBLG50	1/50	54/46	78.4	8502	18499	1.45
PCL-*b*-PBLG70	1/70	34/66	74.3	13320	25515	1.25

[a] Molar ratio of PCL-NH_2 to BLG-NCA

[b] Determined by ^{1}H NMR in $CDCl_3$ solution

[c] Determined by GPC in THF at 30 °C

Characterization

The IR spectra were collected by a Perkin-Elmer FT-IR spectrometer using KBr disks. Elemental analysis was performed on Thermo Electron Flash EA 1112 instrument. ^{1}H NMR spectra were measured on a Varian Mercury-300 NMR spectrometer at room temperature, using $CDCl_3$ as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference. The gel permeation chromatography (GPC) measurement was conducted with a Waters 1515 GPC instrument equipped with a HT4 column (effective molecular weight range: 5000–600,000) and a 2414 differential refractive index detector. THF was used as eluent at the flow rate of 1.0 mL min^{-1} at 30 °C, and the molecular weights were calibrated with polystyrene standards.

Results and discussion

Synthesis of PCL-NH_2

One of the most attractive advantages of microwave heating is its rapid bulk heating compared with the conventional method [22, 23]. In this study, the macroinitiator PCL-NH_2 was prepared by deprotection of a PCL-CH_2CH_2NHBoc, which was successfully obtained by ring-opening polymerization of ε-CL initiated by $HOCH_2CH_2NHBoc$ under microwave irradiation.

The IR spectra of the PCL-NH_2 and PCL-CH_2CH_2NHBoc are shown in Fig. 1. The absence of signal assigned to the secondary amide (1556 cm^{-1}) in the IR spectrum of PCL-NH_2 showed the secondary amide was converted to primary amine, indicating the removal of Boc protecting group.

The ^{1}H NMR spectrum of PCL-NH_2 is shown in Fig. 2. The peak at 1.38, 1.65, and 4.06 ppm are assigned to proton f, e, and g, respectively, in the PCL segment. The peak at 2.31 ppm is assigned to protons b, d in the $H_2NCH_2CH_2O$– and PCL segment, respectively. The peaks at 2.96 and 3.51 ppm are assigned to protons a and c in the $H_2NCH_2CH_2O$– segment, respectively. The peak at 3.65 ppm is assigned to proton h in the end CH_2OH of PCL segment. No methyl proton signal at 1.47 ppm in the ^{1}H NMR spectrum of PCL-NH_2, which indicated that Boc group from

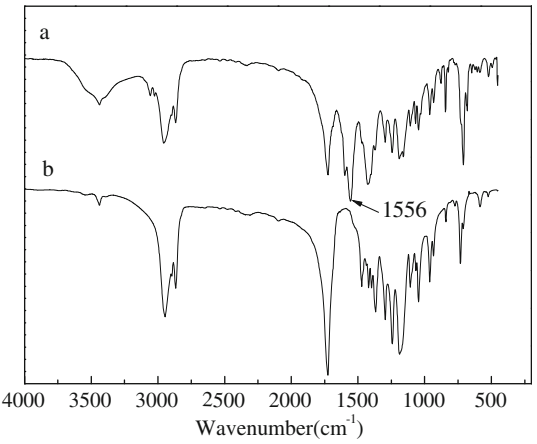


Fig. 1 IR spectra of PCL-CH_2CH_2NHBoc and PCL-NH_2 (*a*) PCL-CH_2CH_2NHBoc, (*b*) PCL-NH_2

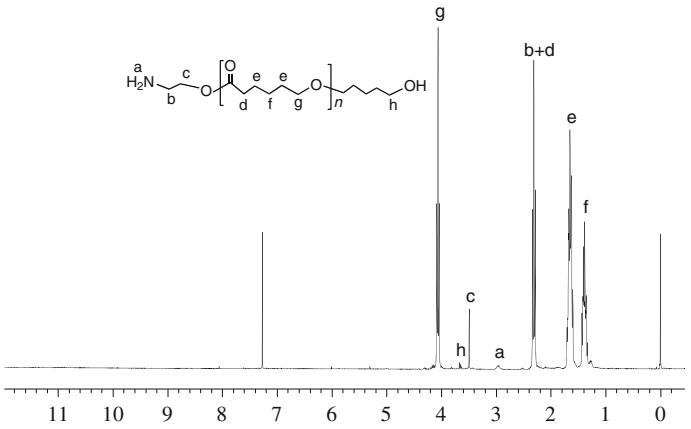


Fig. 2 ^1H NMR spectrum of PCL-NH_2

PCL-CH_2CH_2NHBoc was removed. The results of M_n, M_w/M_n, and yield are shown in Table 1.

The GPC chromatogram of the PCL-NH_2 is shown in Fig. 3. The sample showed unimodal molecular weight distribution. This further indicates that the polymerization is completed successfully and there is no other product.

Synthesis of PCL-*b*-PBLG block copolymer

It is well known that primary amines, being more nucleophilic than basic, can be used as initiators for the ring-opening polymerization of NCA to prepare PAAs, undergoing a nucleophilic addition to the carbonyl group of the NCA [24]. Because PCL-NH_2 contains primary amine group, it can initiate ring-opening polymerization of BLG-NCA to form block copolymer. A series of the block copolymers with various molecular weights were synthesized and the results are summarized in

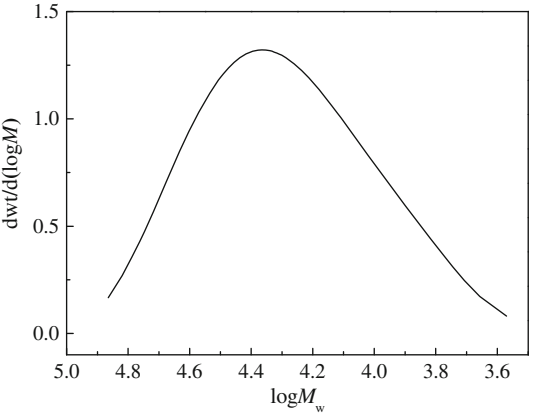


Fig. 3 GPC chromatogram of PCL-NH_2 macroinitiator

Table 1. It was found that the total molecular weights of the copolymers increased with the molar ratio of the feeding monomer BLG-NCA to the initiators.

The IR spectra of PCL-*b*-PBLG block copolymers are shown in Fig. 4. The absorption peak at 3291 cm^{-1} was assigned to ν_{NH} stretch vibration, and the typical amido absorption bands I and II were observed at 1625 and 1522 cm^{-1}, respectively, indicating the formation of the polypeptide segment. The absorptions at 697 and 734 cm^{-1} from the phenyl group were characteristic of the PBLG segment carrying protection groups. No carbonyl absorptions of BLG-NCA at 1863 and 1768 cm^{-1} appeared in the spectra of all products, which indicated that no BLG-NCA residue co-existed in the polymer samples.

The ^1H NMR spectrum of the PCL-*b*-PBLG30 block copolymer is shown in Fig. 5. The peaks at 7.26, 5.07, 2.03, and 1.65 ppm are assigned to protons a, b, c, and d, respectively, in the PBLG segment. The peak at 4.06 ppm was assigned to protons l and f in the PCL and PBLG segments. The peak at 2.31 ppm was assigned to protons g, h, and i in the –NH–CH_2CH_2O– and PCL segments, respectively. The peaks at 1.38 and 1.25 ppm are assigned to protons j and k in the PCL segment. The

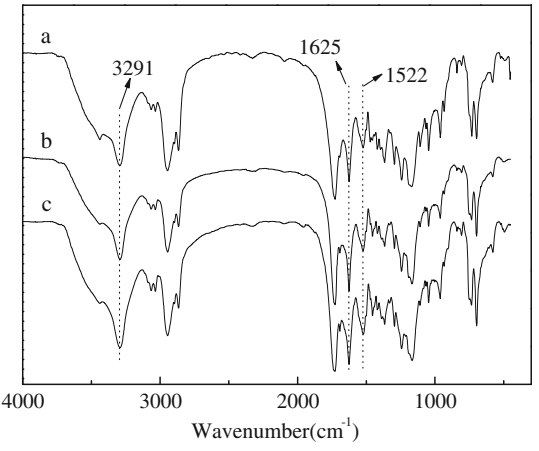


Fig. 4 IR spectra of PCL-*b*-PBLG block copolymers (*a*) PCL-*b*-PBLG30, (*b*) PCL-*b*-PBLG50, and (*c*) PCL-*b*-PBLG70

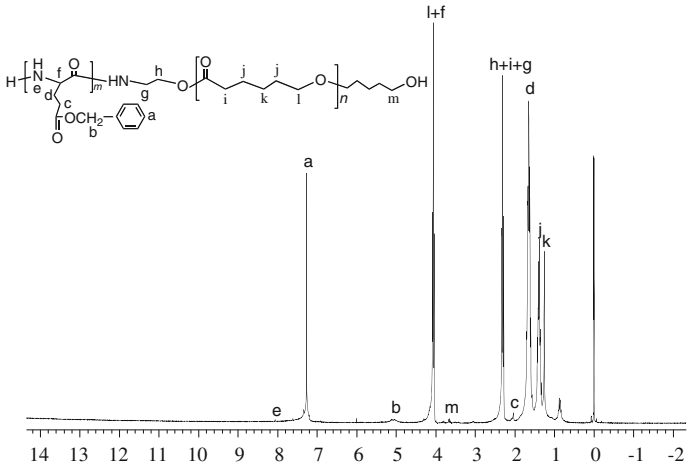


Fig. 5 ^{1}H NMR spectrum of PCL-*b*-PBLG30 block copolymer

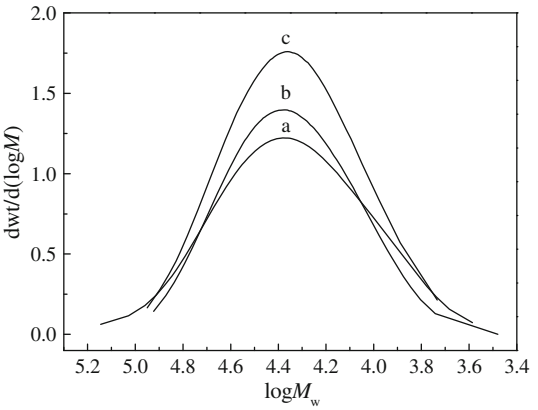


Fig. 6 GPC chromatograms of PCL-*b*-PBLG block copolymers (*a*) PCL-*b*-PBLG30, (*b*) PCL-*b*-PBLG50, and (*c*) PCL-*b*-PBLG70

peak at 8.06 ppm was assigned to proton of e in the copolymer. The peak at 3.65 ppm is assigned to proton m in the end CH_2OH of PCL segment [11, 25]. No additional peaks were detected in the spectrum, implying the block copolymer prepared.

The GPC chromatograms of the block copolymers are depicted in Fig. 6. The three copolymers show a unimodal molecular weight distribution, indicating that the copolymerization is completed successfully and that no homo-polymers were generated. GPC data of the copolymers are listed in Table 1.

Conclusions

The ring-opening polymerization of BLG-NCA could be initiated by PCL-NH_2 in dichloromethane to afford the PCL-*b*-PBLG biodegradable block copolymer.

Characterizations using IR, ^{1}H NMR, and GPC had confirmed the designed structure. The PCL-*b*-PLG amphiphilic block copolymer can be prepared by catalytic hydrogenation of the PCL-*b*-PBLG block copolymer [26]. Further investigations are in progress.

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