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Synthesis and self-assembly of a novel Y-shaped copolymer with a helical polypeptide arm

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ABSTRACT

A novel biodegradable Y-shaped copolymer, poly(L-lactide)$_2$-*b*-poly(γ-benzyl-L-glutamic acid) (PLLA$_2$-*b*-PBLG), was synthesized by the ring-opening polymerization (ROP) of *N*-carboxyanhydride of γ-benzyl-L-glutamate (BLG–NCA) with centrally amino-functionalized poly(L-lactide), PLLA$_2$-NH$_2$, as a macroinitiator in a convenient way. The Y-shaped copolymer and its precursors were characterized by ^{1}H NMR, FT-IR, GPC, WAXD and DSC measurements. The self-assembly of the PLLA$_2$-*b*-PBLG copolymer in toluene and benzyl alcohol was examined. It was found that the self-assembly of the copolymer was dependent on solvent and on relative length of the PBLG block. For a copolymer with PLLA blocks of 26 in total degree of polymerization (DP), if the PBLG block was long enough (e.g., DP = 54 or more), the copolymer/toluene solution became a transparent gel at room temperature. In benzyl alcohol solution, only PLLA$_2$-*b*-PBLG containing ca. 190 BLG residues could form a gel; those with shorter PBLG blocks (e.g., DP = 54) became nano-scale fibrous aggregates and these aggregates were dispersed in benzyl alcohol homogeneously. Copolymers with short PBLG blocks behaved like a pure PLLA both in toluene and in benzyl alcohol. These experimental results were discussed and explained by virtue of the helical conformation of PBLG and the interactions between the solvents and the PLLA and/or PBLG segments.

1. Introduction

In biology, various kinds of biomacromolecules or biomolecules, including proteins, DNA, and phospholipids can spontaneously assemble into a variety of smart nanomaterial systems through well-controlled inter- and/or intra-molecular interactions, such as electrostatics, hydrogen bonding and hydrophobicity. For example, complex cytoskeletal nanofibrils made of biopolymers are nanofiber architectures [1]. In fact, in recent years, various novel macromolecules have been designed and prepared and a variety of aggregates of nano-to-micrometer scale were formed from them [2–5]. For example, sequence controlled oligopeptides can self-assemble into nanostructured fibers, which have been investigated intensively [6,7]. Thus, self-assembly of macromolecules is a very useful means of creating nanostructured materials with tunable properties, and has attracted considerable attention in materials science and biomimetic research [8–10].

Proteins are necessary for human beings. They are known to form α-helices or β-sheets as their fundamental secondary motifs via intra- and intermolecular interactions between the functional groups of residual amino acids. Compared to natural proteins, synthetic polypeptides offer more advantages in stability and processability. Great efforts have been made to incorporate proteins or polypeptides into synthetic materials [11–13]. Compared with those block copolymers without polypeptide blocks, polypeptide-containing ones can simulate not only the shape of natural aggregates, but also their biological performances. Recently, Manners et al. discovered the gelation of the diblock copolymer PBLG-*block*-(random coil polymer) in toluene through a self-assembled nanoribbon mechanism, which is distinct from the current understanding of PBLG self-assembly [14]. It is well known that poly(γ-benzyl-L-glutamate) (PBLG) as a kind of synthetic polypeptide assumes an α-helical conformation in solution or solid state, which leads to a rigid rod structure [15]. The intrinsic rigidity of PBLG results in its unique solution behavior such as thermoreversible gelation and lyotropic liquid-crystalline properties [16,17]. Therefore, as rigid rods, incorporation of α-helical PBLG chains into copolymers is expected to bring about new aggregate structures.

Poly(L-lactide) (PLLA) is a well-known synthetic biodegradable polymer used in surgical repair, carriers in drug delivery, and temporary matrixes or scaffolds in tissue engineering due to its biodegradability, biocompatibility, high mechanical properties, and excellent shaping and molding properties [18,19]. Its combination with peptide blocks can modify the degradation pattern of the polymers because peptidase is required to hydrolyze the peptide

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bonds. The copolymers consisting of both polypeptides and biodegradable polyesters have been studied rarely [20–22]. Moreover, those studied so far are almost linear shaped copolymers. Recently, Lecommandoux et al. synthesized a Y-shaped block copolymer, polystyrene-*b*-(poly(glutamic acid))$_2$, and investigated its self-assembly in bulk. For copolymers, introducing branching points will perturb the conformational entropy of copolymer chains, and consequently, will bring about unique phase-separation behavior either in bulk or in solution compared to the traditional linear copolymers [23,24]. Increasing interest has been given to the preparation of a variety of star copolymers with varying arm number, chemical composition, and chain topology because of their potential applications in nanoscience and nanotechnology [25,26]. Herein, we synthesized a novel Y-shaped copolymer, poly(L-lactide)$_2$-*b*-poly(γ-benzyl-L-glutamic acid) (PLLA$_2$-*b*-PBLG), with a helical polypeptide arm PBLG by the ring-opening polymerization (ROP) of *N*-carboxyanhydride of γ-benzyl-L-glutamate (BLG–NCA) with centrally amino-functionalized polylactide PLLA$_2$-NH$_2$ as a macroinitiator. The PLLA$_2$-NH$_2$ was obtained through ROP of L-lactide in the presence of stannous octoate with 2-amino-1,3-propanediol as an initiator in which the amine group was protected. The PBLG segments can form rigid aggregates in dilute solution of toluene or benzyl alcohol because of its α-helical structure. The PLLA blocks may crystallize or remain solvated in these solvents. Thus, this block copolymer may show unique properties in comparison with common coil–coil or coil–rod type copolymers. Its self-assembly properties in benzyl alcohol and toluene were investigated. Gel and nano-scale fibrous aggregates were obtained under different conditions.

2. Experimental section

2.1. Materials and methods

2.1.1. Materials

L-Lactide (LLA) was purchased from PURAC Biochem bv Gorinchem and recrystallized from ethyl acetate for three times. BLG–NCA was prepared according to Daly's method [27]. 2-Amino-1,3-propanediol (Tokyo Chemical Industry Co., Ltd.) was used without further purification. 33 wt% Solution of HBr in HAc was supplied by Acros. Benzyloxycarbonyl chloride and trifluoroacetic acid were purchased from GL Biochem (Shanghai) Ltd. Tetrahydrofuran (THF) was dried and distilled in the presence of sodium before use. Chloroform was refluxed over CaH_2 and distilled under nitrogen.

2.1.2. Synthesis of 2-benzyloxycarbonylamino-1,3-propanediol

10 g 2-Amino-1,3-propanediol was dissolved in a 10% solution of Na_2CO_3 in water. Dioxane (150 ml) was then added, and the mixture was cooled to 0 °C. Benzyl chloroformate (15 ml) was added dropwise. After 3 h, the mixture was allowed to react at the room temperature for 12 h. Then the mixture was diluted with large amount of water, and was extracted with ethyl acetate. The crude product was recrystallized from ethyl acetate to yield a white needle-like crystalline solid. Yield: 95%. ^{1}H NMR (DMSO-d_6, ppm): 3.3–3.5 (–CH(C**H**$_2$)$_2$–), 4.5 (–O**H**), 5.0 (–C**H**$_2$–Ph), 6.8 (–N**H**–), 7.3 (–C$_6$**H**$_5$).

2.1.3. Synthesis of the benzyloxycarbonyl(Z)amino group bearing poly(L-lactide) PLLA$_2$-NH-Z

The polymer PLLA$_2$-NH$_2$ was prepared by the ROP of L-lactide in the presence of an initiator (2-benzyloxycarbonylamino-1,3-propanediol) and stannous octoate (Sn(Oct)$_2$). Firstly, given amounts of initiator, L-lactide, toluene (25 wt%), and Sn(Oct)$_2$ (1‰ in mole) were added into a dried glass reactor already flame-dried and nitrogen-purged three times, and then the sealed reactor was maintained at 110 °C for 12 h. The product was dissolved in chloroform and precipitated with an excess of diethyl ether to give a white product. The product was washed with methanol several times. Yield: 91.3%. The DP (degree of polymerization) value of PLLA$_2$-NH-Z was calculated from ^{1}H NMR. The copolymers with different DPs could be obtained by adjusting the feed ratio of LLA to the initiator. The results are shown in Table 1.

2.1.4. Synthesis of amino-bearing poly(L-lactide), PLLA$_2$-NH$_2$

The benzyloxycarbonyl (Z) group on PLLA$_2$-NH-Z was removed by reacting with 4 equiv of HBr (in HAc, C = 33%) with respect to the amino group in CF_3COOH (0.1 g/ml) at 0 °C for 1.5 h. The product was precipitated with an excess of diethyl ether to get a white solid and was dried in vacuum at room temperature for 48 h. Yield: 89.2%. The results are shown in Table 1.

2.1.5. Synthesis of Y-shaped copolymers PLLA$_2$-b-PBLG

In a dried flask, given amounts of PLLA$_2$-NH$_2$ and BLG–NCA were dissolved in dried chloroform (10 wt%) and the solution was stirred for 72 h at 30 °C. The product mixture was precipitated with an excess of a mixture of acetic acid and methanol (1:3, v/v) under vigorous stirring to give a white solid while the unreacted PLLA$_2$-NH$_2$ remained dissolved in the solution. Then purified PLLA$_2$-*b*-PBLG was gained under vacuum at 40 °C for 24 h. Yield: 87.4%. The DP value of PBLG was calculated from ^{1}H NMR. The results are summarized in Table 2.

2.1.6. Measurements of the block copolymers

^{1}H NMR spectra were measured in DMSO-d_6 at room temperature (20 ± 1 °C) by an AV-300 NMR spectrometer from Bruker. FT-IR spectra were recorded on a Bio-Rad Win-IR instrument. Gel permeation chromatography (GPC) measurements were conducted with a Waters 410 GPC with tetrahydrofuran (THF) as eluent (flow rate: 1 ml/min, at 35 °C). The molecular weights were calibrated against polystyrene (PS) standards. DSC curves were recorded on a Q100 DSC instrument (TA) under N_2 atmosphere at a rate of 50 ml/min, scanning from 0 °C to 180 °C at a rate of 10 °C/min. One-dimensional (1D) Wide-angle X-ray scattering (WAXD) measurements (Rigaku, D/max 2500 V PC X-ray scattering) were carried out with a Cu K$_\alpha$ radiation ($\lambda = 0.154$ nm). The 2θ scanning rate was 4°/min from 5° to 60°. Selected voltage and current were 40 kV and 200 mA, respectively. The X-ray was calibrated using α-Al2O3 crystals with a known crystal diffraction at $2\theta = 28.47°$.

2.1.7. Preparation of polymer solutions

All copolymers were directly dissolved in filtered benzyl alcohol or toluene in a sealed vial, and the vial was heated until the mixture became homogeneous and then annealed for 1 h before being cooled to room temperature to allow equilibrium to be reached.

2.1.8. Characterization of segregates in benzyl alcohol

Transmission electron microscopy (TEM) measurements were performed on a JEOL JEM-1011 electron microscope operating at an

Table 1
Molecular weight characterization of PLLA$_2$-NH-Z and PLLA$_2$-NH$_2$.

Polymers	M_n[a]	M_n[b]	DP[c]	M_n[d]	M_w/M_n[d]
PLLA$_2$-NH-Z(1)	4000	3700	26	7020	1.16
PLLA$_2$-NH$_2$(1)	–	–	26	6900	1.14
PLLA$_2$-NH-Z(2)	5000	4900	34	10,000	1.21
PLLA$_2$-NH$_2$(2)	–	–	34	9990	1.22

[a] Number average molecular weight calculated from feed composition.
[b] Number average molecular weight determined by ^{1}H NMR in DMSO solution.
[c] Total degree of polymerization of PLLA in a unit of lactide residue calculated from the M_n data determined by ^{1}H NMR.
[d] Number average molecular weight, determined by GPC in THF.

Table 2
Molecular weight characterization of the Y-shaped PLA_2-*b*-PBLG.

Polymers	$PLLA_2$-NH_2			PBLG	
	Macroinitiator	M_n[a]	DP[b]	DP[a]	M_n[a]
$PLLA_2$-*b*-PBLG(1)	$PLLA_2$-NH_2(1)	3700	60	54	11,800
$PLLA_2$-*b*-PBLG(2)	$PLLA_2$-NH_2(1)	3700	100	190	41,600
$PLLA_2$-*b*-PBLG(3)	$PLLA_2$-NH_2(2)	4900	10	8	1750

[a] Calculated from ^{1}H NMR.
[b] Calculated from feed composition.

acceleration voltage of 100 kV. A drop of the dilute benzyl alcohol solution was deposited onto a copper grid for about 5 min and then was blotted up with a piece of filter paper. At last, the sample was put at room temperature. For the gel samples, a copper grid was placed on the gel for 10 s, and then removed to dry at room temperature.

3. Results and discussion

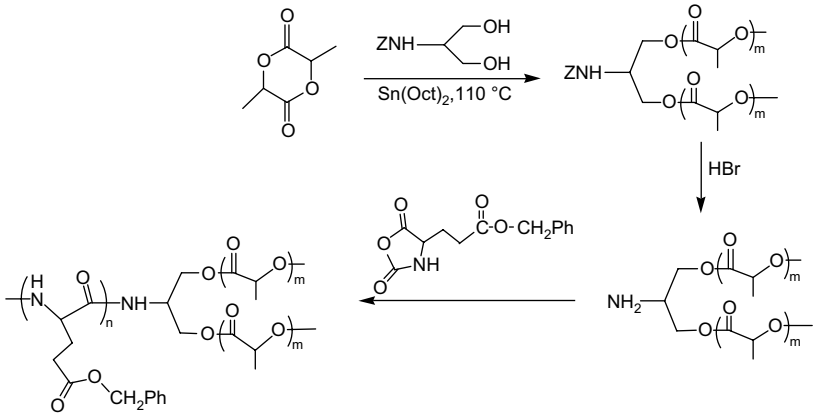
3.1. Synthesis and characterization of the copolymer

For synthesis of the Y-shaped copolymer $PLLA_2$-*b*-PBLG, the key is the synthesis of a PLLA, which contains a primary amine group at the middle of its molecular chain. Once it is obtained, the third arm of PBLG can be attached via the ROP of BLG–NCA initiated by the NH_2 on PLLA because NH_2-initiated ROP of amino acid *N*-carboxyanhydride (NCA) is an efficient and well established method for poly(amino acid) preparation [28,29]. Recently, Schlaad and Dimitrov elaborated conditions for the truly controlled *N*-carboxyanhydrides block copolymer synthesis through avoiding the "activated monomer" process [30]. However, it is difficult to introduce an amino group into a PLLA chain, for example, to convert its end OH group directly into NH_2, mainly because its weak aliphatic ester bonds are easy to be broken down under strong reaction conditions such as severe acid or severe alkali. Höcker et al. [20] successfully synthesized MPEG–PLA-NH_2 by end-capping PLA with *N*-*tert*-butoxycarbonyl (Boc) phenylalanine and then removing the Boc group. Our group further used MPEG–PLLA-NH_2 as a macromolecular initiator to get triblock copolymers MPEG-*b*-PLLA-*b*-PBLG and MPEG-*b*-PLLA-*b*-PZLL [19,22]. Moreover, there is another method which is first described by Höcker group. Amino-functionalized poly(L-lactide) is synthesized through the ROP of L-lactide with zinc *tert*-butoxycarbonylaminopropanoxide as the initiator and subsequent deprotection under acidic environment. The initiator is prepared by the reaction of diethylzinc and Boc-aminopropanol. Because of intrinsic instability, it must be used immediately after the preparation. In the present study, 2-benzyloxycarbonylamino-1,3-propanediol is used as the initiator and stannous octoate ($Sn(Oct)_2$) is used as the catalyst to prepare the two PLLA blocks. The two OH end-groups of 2-benzyloxycarbonylamino-1,3-propanediol are equivalent in reactivity, therefore it is believed that the two PLLA chains are of the same length. Utilization of the 2-benzyloxycarbonylamino group is for incorporating NH_2 group in between the two PLLA blocks. The benzyloxycarbonyl (Z) group is chosen as the amino protective group instead of Boc, because Boc group is not stable enough at 110 °C during LLA polymerization [31].

The synthetic route for $PLLA_2$-*b*-PBLG is outlined in Scheme 1. The $PLLA_2$-NH-Z is synthesized with high conversion directly via the ROP of LLA in the presence of 2-benzyloxycarbonylamino-1,3-propanediol and $Sn(Oct)_2$ in toluene solution. The characteristics of the synthesized polymers are shown in Table 1. The block lengths of $PLLA_2$ can be adjusted by changing the molar ratio of LLA to initiator. The molecular weights and their polydispersity of $PLLA_2$-NH-Z are characterized by theoretical calculation, ^{1}H NMR and GPC. Typical signals of both $PLLA_2$ and benzyloxycarbonyl units are detected by ^{1}H NMR as shown in Fig. 1A. The peaks marked with letters a and b can be assigned to the protons in $PLLA_2$ repeat-units, i.e., a at 5.2 ppm (quadruplet) to –C(O)C**H**(CH_3)O–, b at 1.4 ppm (doublet) to –C(O)CH(C**H$_3$**)O–. The peaks c, d (at 4.0–4.2 ppm), e (at 5.0 ppm), and f (at 7.3 ppm) are assigned to the protons of benzyloxycarbonyl group. DP_{PLLA_2} is obtained from the integral ratio of C_6**H$_5$**– (f at 7.3 ppm) to –C(O)C**H**(CH_3)O–C(O)C**H**(CH_3)O– (a at 5.2 ppm) in the ^{1}H NMR spectrum of $PLLA_2$-NH-Z, by means of the formula $DP_{PLLA_2} = 5a/2f$. The GPC traces of all polymers show a unimodal distribution as shown in Fig. 3(A), which further indicates that the $PLLA_2$-NH-Z is successfully obtained.

As it is known, the benzyloxycarbonyl protective groups can be removed by acidolysis with a 33% solution of HBr in HAc [32]. With this method, $PLLA_2$-NH-Z is reduced to $PLLA_2$-NH_2. The removal of the Z group is confirmed by ^{1}H NMR as shown in Fig. 1B, because the benzyl peaks at 5.0 ppm and 7.3 ppm disappear completely. The GPC trace of $PLLA_2$-NH_2 in Fig. 3B looks similar to that in (A). The molecular weight characteristics are shown in Table 1. The molecular weights and their distribution do not change obviously, indicating that the PLLA chains do not degrade appreciably during the deprotection process. Therefore, the designed $PLLA_2$-NH_2 is successfully synthesized.

The primary amines can be used as initiators for the ROP of NCA to prepare poly(α-amino acid)s, undergoing a nucleophilic addition to the C-5 carbonyl group of the NCA [28]. Therefore, the $PLLA_2$-NH_2 is used as a macromolecular initiator to synthesize Y-shaped copolymers $PLLA_2$-*b*-PBLG according to Scheme 1. The results are summarized in Table 2. The total DP of the two PLLA



Scheme 1. Synthesis of Y-shaped copolymer $PLLA_2$-*b*-PBLG.

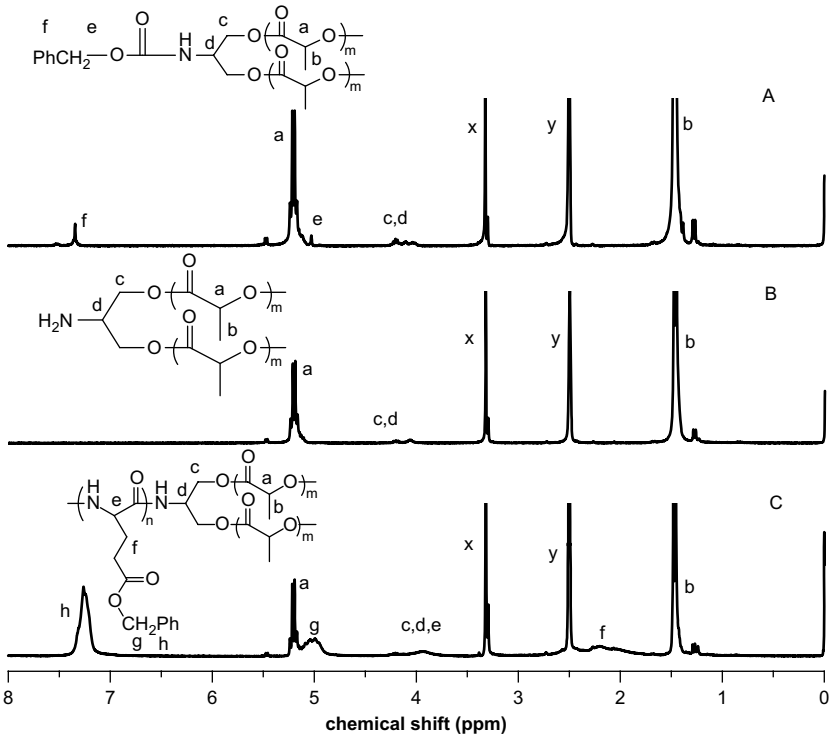


Fig. 1. ^{1}H NMR spectra and their assignments of PLLA$_2$-NH-Z(1) in Table 1 (A), PLLA$_2$-NH$_2$(1) in Table 1 (B), and PLLA$_2$-*b*-PBLG(1) in Table 2 (C) in DMSO-d_6. x and y are solvent peaks.

blocks varies between 26 and 34, and the DP of PBLG varies between 8 and 190.

The ^{1}H NMR spectrum of the PLLA$_2$-*b*-PBLG block copolymer is shown in Fig. 1C. The peak h at 7.2 ppm is attributed to the benzene ring of the protecting group. The peaks at 4.9, 4.0, and 2.0–2.4 ppm are assigned to protons on the PBLG main-chain. The peaks at 5.2 and 1.4 ppm are assigned to protons of the PLLA blocks. DP$_{PBLG}$ in the copolymer is obtained from the integral ratio of –C(O)C**H**-(CH$_3$)O–C(O)C**H**(CH$_3$)O– (a at 5.2 ppm) to –C$_6$**H$_5$**– (h at 7.2 ppm) in the ^{1}H NMR spectrum of PLLA$_2$-*b*-PBLG, i.e., DP$_{PBLG}$ = 2 DP$_{PLLA_2}$ h/5a.

The structure of the copolymer PLLA$_2$-*b*-PBLG is also confirmed by its IR spectra (Fig. 2). The absorption peak at 3292 cm^{-1} is assigned to ν_{NH} of PBLG, and the peaks at 1653 cm^{-1} (amide I) and 1548 cm^{-1} (amide II) are attributed to the amide group, indicating the formation of the α-helical polypeptide block. The absorptions at 697 and 749 cm^{-1} from the phenyl group are characteristic of the PBLG block carrying protective groups. The peaks at 1734 cm^{-1} (ν_{CO}) and 1087 cm^{-1} (δ_{C-O-C}) are corresponding to PLLA blocks.

The GPC trace of the copolymer (Fig. 3C) shows a unimodal shape. It further indicates that the copolymerization has been completed successfully and no homopolymerization occurred. However, this method cannot be used to determine molecular weights or polydispersity indices of the block copolymers, because self-assembly of the diblock copolymer may exist [4]. The DSC data are shown in Table 3. In comparison to the linear PLLA, the Y-shaped PLLA$_2$ shows lower T_g and T_m. This may be due to its limited length (DP = 13–17) and branched structure. Moreover, presence of the PBLG block is verified by a small peak with a typical low enthalpy change observed around 100 °C (T_{LC}). This observed transition is irreversible and only occurred during the first heating run; it is attributed to an irreversible change from a 7/2 to an 18/5 α-helical conformation [33]. At higher temperature, no other transition could be observed for PBLG until its degradation [34,35]. It can be seen that T_g of the PLLA$_2$-*b*-PBLG copolymers shifts slightly to a higher temperature with increasing chain length of PBLG, probably because the mobility of PLLA$_2$ chains is constrained. Fig. 4 shows a set of 1D WAXD results for the block copolymer PLLA$_2$-*b*-PBLG(1) in Table 2 and the homopolymer. The WAXD pattern of the copolymer is identical to that of the PLLA homopolymer, expect a diffraction peak near $2\theta = 6°$, which corresponds to the distance between PBLG helices (d = 1.5 nm) [15]. It implies that there exist both crystalline PLLA micro-domains and PBLG helices in the aggregates, which is in agreement with the DSC results.

3.2. Self-assembly property of the copolymers

The copolymer is composed of both polypeptide and poly(L-lactide) blocks and thus is expected to exhibit unique assembling property. As it is known, due to α-helical secondary structure, the PBLG segments can form rigid aggregates in dilute solution of

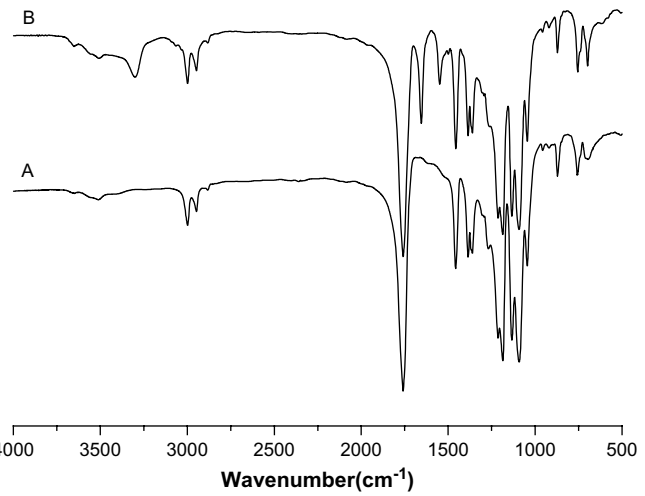


Fig. 2. IR spectra of (A) PLLA$_2$-NH-Z(2) in Table 1, and (B) PLLA$_2$-*b*-PBLG(3) in Table 2.

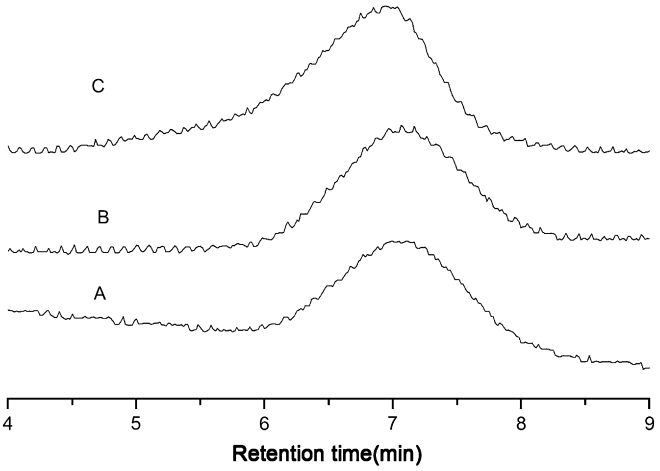


Fig. 3. The GPC traces of $PLLA_2$-NH-Z(2) in Table 1 (A), $PLLA_2$-NH_2(2) in Table 1 (B), and $PLLA_2$-*b*-PBLG(3) in Table 2 (C).

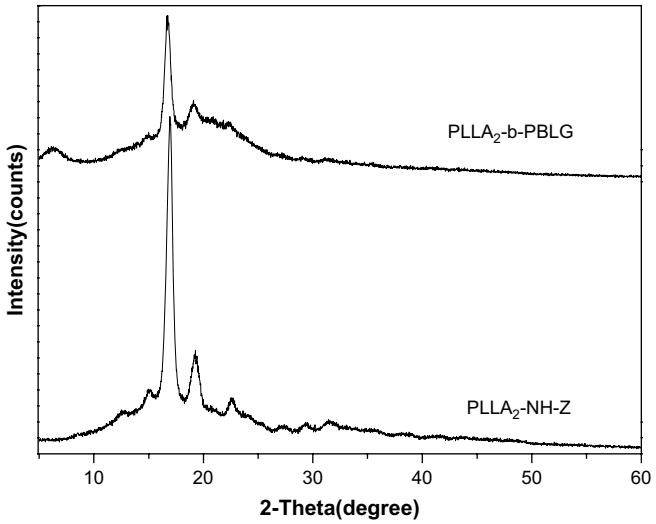


Fig. 4. WAXD patterns of the homopolymer $PLLA_2$-NH_2-Z(1) in Table 1 and the block copolymer $PLLA_2$-*b*-PBLG(1) in Table 2.

toluene. Meanwhile, the PLLA blocks may crystallize or remain solvated in toluene [36]. In fact, the copolymer $PLLA_2$-*b*-PBLG(1) can dissolve in hot toluene (at about 50 °C). When the uniform solution (10 mg/ml) in toluene is cooled to room temperature (ca. 22 °C), a transparent gel is formed (Fig. 5A, inset). Fig. 5A presents TEM image of the dried gel formed from $PLLA_2$-*b*-PBLG(1)/toluene solution. It is of fibrous structure. Each individual fiber has a width of about 30 nm and a length of several tens of micrometers. This gel morphology can be explained as follows. Firstly, any gel formation is related to a chemical or physical crosslinking. Manners et al. have noted that the gelation of diblock copolymers of the type PBLG-*block*-(random coil polymer) in toluene (a helicogenic solvent) occurred through a self-assembled nanoribbon mechanism [14]. For our system, structure of the Y-shaped copolymers is similar to copolymer PBLG-*block*-(random coil polymer). Thus, it can be suggested that the fibrous morphology observed in Fig. 5A is actually of ribbon-like structure (B). That is to say, during solution cooling, the PBLG segments in α-helical conformation further aggregate to form such ribbons and these ribbons are dispersed in the solution homogeneously to provide mechanical strength for the whole gel. Meanwhile, because toluene is a good solvent for PLLA at both elevated and room temperatures, the PLLA segments remain dissolved in toluene at room temperature. On the one hand, these PLLA segments are distributed homogeneously in the whole solution, on the other hand, they cannot leave the PBLG ribbons because the PLLA and PBLG segments have been chemically combined together. Therefore, the PLLA segments assume a coil conformation in the gel. During the TEM sample preparation (toluene evaporation), they may crystallize or remain non-crystalline. In order to observe whether the PLLA is in crystalline state, electron diffraction (ED) is performed on the TEM sample. No crystalline ED patterns are obtained (data not shown). It implies that the PLLA segments are in a non-crystalline state in the TEM sample and PLLA crystallization has not taken place. This result provides direct evidence for the coil-like structure of PLLA in the gel and for the following conclusion that the observed aggregates in Fig. 5A are composed of PBLG α-helices and PLLA segments which are deposited on the PBLG α-helices. This conclusion has another experimental support: the width of the ribbons in Fig. 5A is ca. 30 nm, while the length of the PBLG helix in $PLLA_2$-*b*-PBLG(1) is calculated to be 8.1 nm ($L_{helix} = N_{PBLG} \times 0.15$ nm, where N_{PBLG} is the average number of residues in the PBLG helix determined by ^{1}H NMR [19], see Table 2, Fig. 5B). The difference between the both is attributed to the deposition of PLLA chains on the surface of PBLG aggregates during the TEM sample preparation (solvent evaporation). Fig. 5A further indicates that even in the dried gel, the ribbons are separated from each other, implying that the PBLG chains span the whole gel with the help of coil-like PLLA chains which are strongly solvated by toluene.

To prove the above point of view, the dried gel sample on the copper grid is heated to 80 °C for 1 h. The ED is performed after the sample is cooled to room temperature. As shown in Fig. 5C, the ED is composed of six intense diffraction arcs, which are corresponding to 110 and 200, revealing the existence of PLLA crystals. This observation provides powerful support for the following explanation that the room temperature evaporation is responsible for the non-crystalline state of PLLA in the dried gel. Because the T_g of PLLA is about 60 °C, it is difficult for PLLA segments to crystallize at room temperature even from its toluene solution.

Furthermore, the effects of polymer composition and chain length are also investigated. With a longer PBLG segment in sample $PLLA_2$-*b*-PBLG(2) ($DP_{PBLG} = 190$), a gel is also formed when its solution (5 mg/ml or higher) is cooled from about 70 °C to room temperature. However, the sample $PLLA_2$-*b*-PBLG(3) ($DP_{PBLG} = 8$) can dissolve in toluene at about 50 °C, but remains in solution state when the solution is cooled to room temperature no matter what solution concentration (in the range of 5–10 mg/ml) is used. It implies that there is a critical PBLG length for the gel formation of $PLLA_2$-*b*-PBLG copolymers. Below this length, e.g., in $PLLA_2$-*b*-PBLG(3), on the one hand, the PBLG segment is too short to form a stable α-helix and the ribbon-like aggregates cannot be formed, on the other hand, the PLLA segments are highly soluble in toluene at room temperature and thus the short PBLG segments are brought into the solution because they are chemically linked to the PLLA chains.

Table 3
DSC data of the polymers.

Polymers	T_g/°C	T_m/°C	T_{LC}/°C
Linear PLLA-1[a]	50.0	134.2	–
$PLLA_2$-NH-Z(1)	48.3	120.0	–
$PLLA_2$-*b*-PBLG(1)	54.2	116.7	98.7
$PLLA_2$-*b*-PBLG(2)	53.8	118.0	92.9
Linear PLLA-2[b]	53.7	145.7	–
$PLLA_2$-NH-Z(2)	51.3	140.5	–
$PLLA_2$-*b*-PBLG(3)	53.0	134.7	79.8

[a] Linear PLLA-1, with the same M_n as $PLLA_2$-NH-Z(1).
[b] Linear PLLA-2, with the same M_n as $PLLA_2$-NH-Z(2).

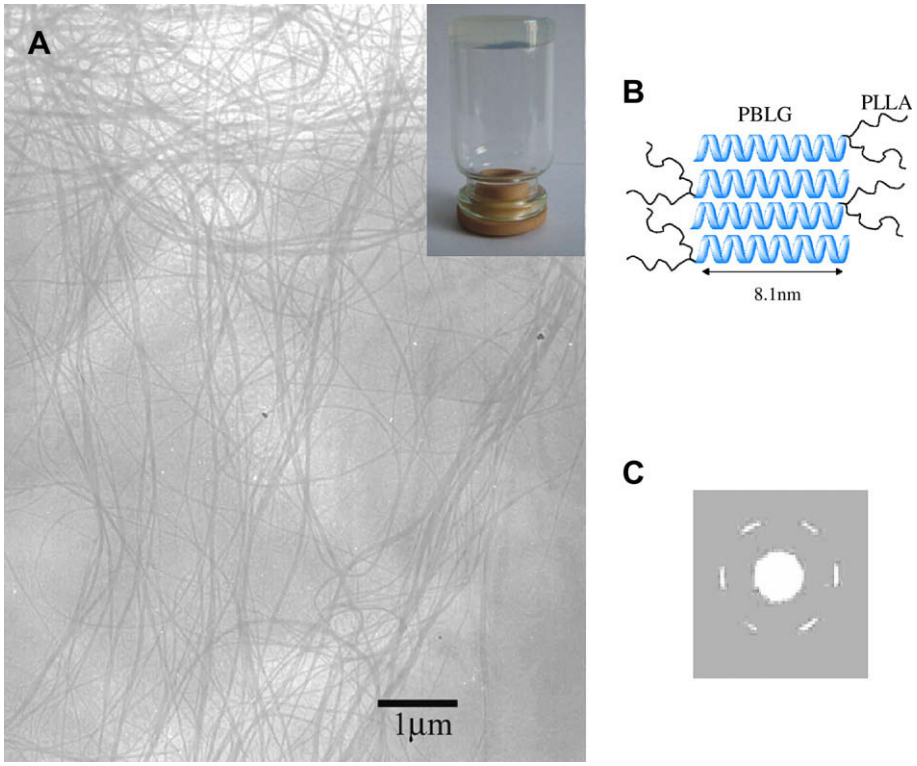


Fig. 5. (A) The TEM image of $PLLA_2$-*b*-PBLG(1) (10 mg/ml)/toluene solution after solvent evaporation. The inset shows the transparent gel. (B) Schematic graph of the dried gel. (C) The ED image of sample A on the copper grid which has been heated to 80 °C for 1 h.

Similar experiments are performed by using benzyl alcohol as solvent. It is known that PBLG can also form rigid rods in dilute solution of benzyl alcohol. Although benzyl alcohol is not as good as toluene for PLLA, all the $PLLA_2$-*b*-PBLG samples can dissolve in hot benzyl alcohol (ca. 50 °C for $PLLA_2$-*b*-PBLG(1) and $PLLA_2$-*b*-PBLG(3), ca. 70 °C for $PLLA_2$-*b*-PBLG(2)). When their benzyl alcohol solutions are cooled to room temperature, following results are obtained: instead of a gel, $PLLA_2$-*b*-PBLG(1) solution (10 mg/ml) remains as a bluish solution; $PLLA_2$-*b*-PBLG(2) solution (5 mg/ml or higher) becomes a gel (Fig. 6B, inset); and $PLLA_2$-*b*-PBLG(3) solution becomes a little bit opaque. The TEM images are shown in Fig. 6A and B for the dried $PLLA_2$-*b*-PBLG(1) solution and $PLLA_2$-*b*-PBLG(2) gel, respectively. In Fig. 6A, individual dendritic aggregates are observed. They are composed of small fibers of about 30 nm in diameter. Their total size is 1–2 μm. They are too small to span the whole solution. Therefore, the solution does not become a gel. The fibers in Fig. 6B are much longer than those in (A). They are imagined to be the supporting frame of the gel. The ED is performed on the dendritic aggregates in Fig. 6A. As shown in Fig. 6C, the ED pattern is two diffraction rings composed of many strong diffraction spots. From the calculation, the Bragg spacing of the inner ring is 1.6 nm, which may belong to the distance between PBLG helices

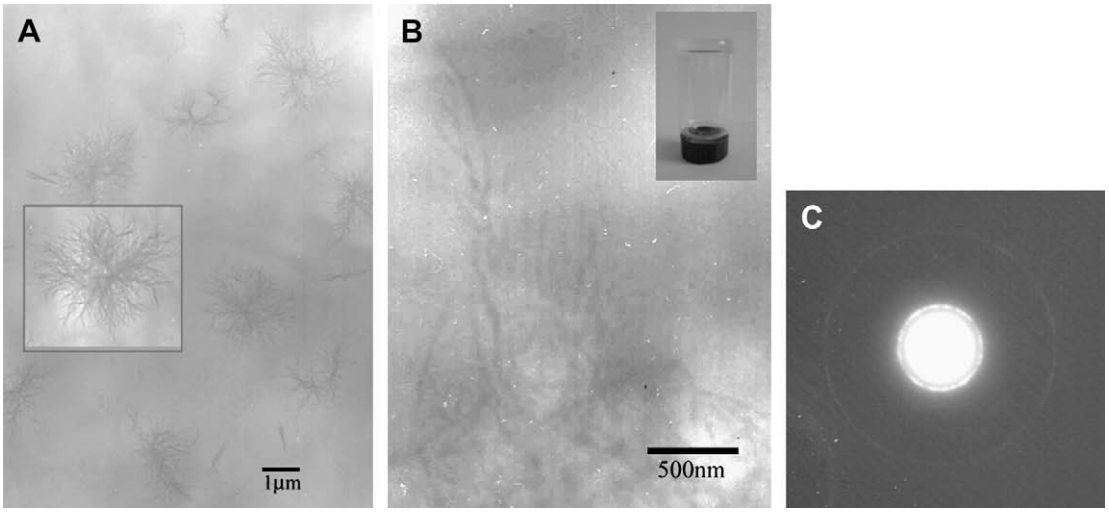


Fig. 6. (A) TEM image of the dried $PLLA_2$-*b*-PBLG(1)/benzyl alcohol solution (10 mg/ml). The inset in (A) is the magnification of the aggregates. (B) TEM image of the dried $PLLA_2$-*b*-PBLG2 (5 mg/ml)/benzyl alcohol gel. The inset shows the transparent gel. (C) ED image of the dried $PLLA_2$-*b*-PBLG(1)/benzyl alcohol solution (10 mg/ml).

($d = 1.5$ nm). The Bragg spacing of the outer ring is similar to the ED in Fig. 5B, implying the existence of the PLLA polycrystals. Existence of these PLLA crystallites and the small size of the dendritic aggregates provide evidence for the crystallization of PLLA segments during solution cooling, because if it was not the case, the PLLA chains would have remained solvated as in the $PLLA_2$-*b*-PBLG(1)/toluene gel and the system would have been a gel, not a nano-aggregate solution. In other words, because benzyl alcohol is not a good solvent for PLLA, PLLA chains get de-solvated and crystallized during cooling, leading to phase separation between PLLA and benzyl alcohol, and therefore, the whole system behaves as a liquid, not as a gel [36,37]. Furthermore, the individual dendritic aggregates can be seen in the TEM sample and the solution displays bluish appearance, indicating that they are dispersed in the solution homogeneously, not precipitated from the solution. This is attributed to the interactions of the PLLA and PBLG segments with benzyl alcohol solvent. On the one hand, part of PLLA chains are not crystallized and they remain solvated. On the other hand, there are strong π–π interactions between aggregated PBLG helices and benzyl alcohol, because the benzyl groups are surrounding the helices.

The only difference between $PLLA_2$-*b*-PBLG(1) and $PLLA_2$-*b*-PBLG(2) is the length of PBLG block but their benzyl alcohol solutions at room temperature are bluish solution and transparent gel, respectively. Obviously, chain length of PBLG plays a key role. Possible explanations are as follows. (1) The PBLG chains assume helical conformation and the PBLG helices assemble themselves into ribbon-like aggregates. Because of the strong π–π interactions between aggregated PBLG helices and benzyl alcohol, these aggregates display improved solubility in benzyl alcohol and do not precipitate from the solution. (2) Compared with the PBLG block, the PLLA blocks in $PLLA_2$-*b*-PBLG(2) are relatively shorter. Because the PLLA blocks are covalently bound to the PBLG ends, the chain density of PLLA on the surface of PBLG helices or PBLG ribbons is much less than in the case of $PLLA_2$-*b*-PBLG(1). As a result, the PLLA chains crystallize with more difficulty or even cannot crystallize at all. More PLLA segments or all PLLA segments are solvated, leading to gel formation.

As for $PLLA_2$-*b*-PBLG(3), because its PBLG is too short, it behaves like pure PLLA and partially crystallizes in cooled benzyl alcohol as observed.

4. Conclusion

A novel Y-shaped copolymer poly(L-lactide)$_2$-*b*-poly(γ-benzyl-L-glutamate) ($PLLA_2$-*b*-PBLG) is synthesized by ROP of *N*-carboxyanhydride of γ-benzyl-L-glutamate (BLG–NCA) with amino-bearing polymer $PLLA_2$-NH_2 as a macroinitiator. The chemical structure of the block copolymer is confirmed by NMR, FT-IR, GPC, WAXD and DSC. Self-assembly of the copolymers in toluene and benzyl alcohol is described. It is found that the self-assembly of the copolymer was dependent on solvent and on relative length of the PBLG block. For a copolymer with PLLA blocks of 26 in total degree of polymerization (DP), if the PBLG block is long enough (e.g., DP = 54 or more), the copolymer/toluene solution becomes a transparent gel at room temperature. In benzyl alcohol solution, only the $PLLA_2$-*b*-PBLG containing ca. 190 BLG residues can form a gel; those with shorter PBLG blocks (e.g., DP = 54) become nano-scale fibrous aggregates and these aggregates were dispersed in benzyl alcohol homogeneously. Copolymers with short PBLG blocks behave like a pure PLLA both in toluene and in benzyl alcohol. These experimental phenomena are attributed to the helical conformation of PBLG, and to the interactions between the solvents and the PLLA and/or PBLG segments which are responsible for the crystallization or solvation of PLLA segments or for precipitation or dispersion of PBLG α-helices.

Moreover, the deprotection of the PBLG blocks will introduce the COOH groups into the copolymers to obtain COOH-functionalized polymers. Further investigation is undertaken and the application of these copolymers will be published elsewhere.

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