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Synthesis and NMR Characterization of Multi-hydroxyl End-groups PEG and PLGA-PEG Barbell-like Copolymers

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Multi-hydroxyl end-groups poly(ethylene glycol) (PEG) was prepared from PEG and epichlorohydrin. Then, PEG-supported poly(lactic-ran-glycolic acid) $(PLGA)_n$-PEG-$(PLGA)_n$ (n = 1, 2, 4) linear-dendritic barbell-like copolymers were synthesized through direct polycondensation under bulk condition from the multi-hydroxyl end-groups PEG, lactic acid and glycolic acid. Arm numbers were varied, with 2, 4 and 8, by using bis-, tetra-, and octa-hydroxyl end-groups PEG, respectively. The chemical structures, absolute number-average molecular weight, the monomer units per single arm and the molar ratio of hydroxyl acid monomer units of the $(PLGA)_n$-PEG-$(PLGA)_n$ barbell-like copolymers were analyzed by NMR spectroscopy. The result indicated that the structures of the multi-hydroxyl end-groups PEG and $(PLGA)_n$-PEG-$(PLGA)_n$ barbell-like copolymers were consistent with design. Compared with the theoretical values, molecular weights determined by ^{1}H-NMR end-group analysis gave reasonably consistent values, but the values determined by gel permeation chromatography (GPC) were considerably less than theoretical values. The results indicated that $(PLGA)_n$-PEG-$(PLGA)_n$ copolymers have linear-dendritic structures.

Keywords: biodegradable polymer; direct polycondensation; poly(ethylene glycol); barbell-like copolymers; linear-dendritic copolymers

1. Introduction

Linear-dendritic copolymers have hybrid structures which combine two types (linear and dendritic) of macromolecular architectures (1). Combining one or more dendritic moieties with one or more linear chain in a single macromolecule can have a profound effect on the ultimate properties of the hybrid material that results. It is only within the past several years that a significant number of reports of such true hybrid copolymers have appeared (2, 3).

The linear-dendritic copolymers may be divided into five major groups: AB diblock linear-dendritic copolymers containing a linear A block and dendritic B block (4–7), ABA triblock linear-dendritic copolymers containing B as the linear block and A as the dendritic block (8–11), side chain functionalized or dendronized linear-dendritic copolymers (12–14), linear-dendritic star copolymers in which the dendritic blocks have connected to the end of arms of a star polymer (15–18), and multi-arm linear-dendritic block copolymers with dendrimer core and linear polymer arms (19). ABA triblock hybrid linear-dendritic copolymers containing PEG as B block and dendritic as A block have been very well studied because of their interesting properties and applications. The first linear-dendritic diblock copolymer through divergent growth on the PEG as the A block was synthesized by Chapman and co-workers (20). This involved divergent dendritic growth of a poly(lysine) dendritic block from an amine terminated PEO.

In addition, there have been few reports about biodegradable linear-dendritic block copolymers. Kim et al. (21) designed and synthesized a novel linear-dendritic copolymer containing thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAM), hydrophobic and biodegradable poly(L-lactic acid) (PLLA), and hydrophilic poly(L-lysine) (PLL) dendrons by 1,3-dicyclohexylcarbodiimide (DCC) coupling reaction of three generation PLL dendrons and PNIPAAM grafted with PLLA. Klok et al. (22) reported the synthesis and supramolecular organization of a novel class of linear-dendritic block copolymers: the molecules, which were termed rodcoil dendrons, consist of a cholesterol moiety that is attached to L-lysine dendrons of three different generations via a biodegradable oligo(L-lactic acid)$_n$ spacer. Cao and his co-workers synthesized new asymmetric AB_n-shaped amphiphilic diblock methoxy poly(ethylene

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glycol)-*b*-[poly(L-lactide)]$_n$, ($n = 2$, 4, and 8), bridged with dendritic ester linkages (23). They also designed and synthesized a novel symmetric amphiphilic polycationic dendritic poly(L-lysine)-*b*-poly(L-lactide)-*b*-dendritic poly(L-lysine) D_2-LLA_{15}-D_2 bearing two two-generation poly(L-lysine) PLL dendrons D_2 and a central hydrophobic biodegradable poly(L-lactide) block LLA_{15} (24). However, to our best knowledge, there have no any reports about the synthesis of biodegradable ABA-shaped linear-dendritic block copolymers via direct polycondensation.

In this study we designed and synthesized PEG-supported PLGA amphiphilic barbell-like copolymers: linear-dendritic $(PLGA)_n$-*b*-PEG-*b*-$(PLGA)_n$ ($n = 1$, 2, 4) block copolymers were synthesized from bis-, tetra- and octa-hydroxyl-terminated PEG, lactic acid and glycolic acid through direct polycondensation under bulk conditions. This method is advisable because direct polycondensation is easier and cheaper compared with ring-opening polymerization, also the products here have low melting temperature, low melting viscosity and excellent hydrophilic properties. The resultant copolymers were characterized by nuclear magnetic resonance (NMR) technique.

2. Experimental

2.1 Materials

Glycolic acid (GA, 98 wt%) solid was purchased from YiXing GaoJing Chemical Corp. (JiangSu, China). D,L-lactic acid (D,L-LA, 85∼90 wt% aqueous solution) was purchased from TianJin Chemical Corp. (TianJin, China). Poly(ethylene glycol) ($\bar{M}$n = 950∼1050, DP = 21∼23) (PEG1000, bihydroxyl PEG), tetrahydrofuran (THF), epichlorohydrin, potassium (K), potassium hydroxide (KOH), anhydrous magnesium sulfate ($MgSO_4$), stannous chloride ($SnCl_2$), purchased from TianJin Chemical Corp. (TianJin, China) were used without further purification.

2.2 Instrumental Measurements

The NMR spectra were recorded on Mercury-400BB NMR spectrometer (VARIAN) in $CDCl_3$ using TMS as an internal standard. The number-average molecular weight ($\bar{M}$n) and weight-average molecular weight ($\bar{M}$w) were determined with respect to polystyrene standards by gel permeation chromatography (GPC) on a GPCV2000 (Waters) machine at 30°C, THF was used as the mobile phase (flow rate = 1.0 ml/min). Poly(styrene) standard samples were used for calibration.

2.3 Synthesis of Tetra- and Octa-Hydroxyl Polyethylene Glycol

A solution of PEG1000 (30 g, 3×10^{-2} mol) and potassium (2.42 g, 6.2×10^{-2} mol) in 60 mL of THF was added to a 150 mL three-neck flask and the mixture was stirred at 50°C for 10 h under a nitrogen flow. Then epichlorohydrin (16.6 g, 1.8×10^{-1} mol) was added and the resulting mixture was stirred at 50°C for another 10 h. Then the mixture was cooled and the residue was filtered off. The organic phase was washed with water several times, dried with anhydrous magnesium sulfate and the organic solvent was evaporated under vacuum to give PEG bisglycidyl ether.

The obtained PEG bisglycidyl ether and 10 mL of 30% KOH aqueous solution were added to a 150 mL three-neck flask and the mixture was stirred at 80°C for 10 h. Then the mixture was cooled and dissolved in 30 mL of dichloromethane. The organic phase was washed with water several times, dried with anhydrous magnesium sulfate and the organic solvent evaporated under vacuum. PEG with four hydroxyl groups was obtained as a white solid.

The octahydroxyl PEG was synthesized with successive repeating of the above-mentioned procedure substituting tetrahydroxyl PEG for PEG.

2.4 Synthesis of $(PLGA)_n$-PEG-$(PLGA)_n$ Barbell-like Copolymers

A mixture of 2.1 g D,L-lactic acid, 1.8 g glycolic acid, 1.0 g bihydroxyl PEG and $SnCl_2$ was stirred at 165∼210°C for 10∼12 h in a 100-mL three-neck flask under a pressure of 1300 Pa. The resultant copolymer was dissolved in 150 mL of THF and precipitated in a mixture of 200 mL of diethyl ether and 100 mL of petroleum ether. The PLGA-PEG-PLGA copolymer was obtained, it was marked S1.

The four and eight arm PEG-supported PLGA barbell-like copolymers were synthesized with successive repeating of the above-mentioned procedure except PEG was changed to 0.50 g tetrahydroxyl PEG and 0.25 g octahydroxyl PEG, and the products was marked S2 and S3, respectively.

3. Results and Discussion

3.1 ^{1}H-NMR Characterization of Polyethylene Glycol Derivatives

In order to construe the tetra- and octa-hydroxyl poly(ethylene glycol) had been synthesized from PEG and epichlorohydrin, the chemical structure of poly(ethylene glycol) bisglycidyl ether, tetrahydroxyl poly(ethylene glycol) and poly(ethylene glycol) tetraglycidyl ether were characterizied by ^{1}H-NMR, and the results are shown in Figures 1, 2 and 3, respectively.

The average degree of polymerization ($\overline{DP}$) of PEG1000 is 22, therefore, the theoretical intensity ratio of methylene (PEG, 3.6 ppm) resonance to methine (glycidyl, 2.89 ppm) resonance of poly(ethylene glycol) bisglycidyl ether in ^{1}H-NMR spectrum is 44/1. From Figure 1, the real intensity ratio was 31.6/0.7 (45/1), therefore, the epoxide number of poly(ethylene glycol) bisglycidyl ether is 1.96.

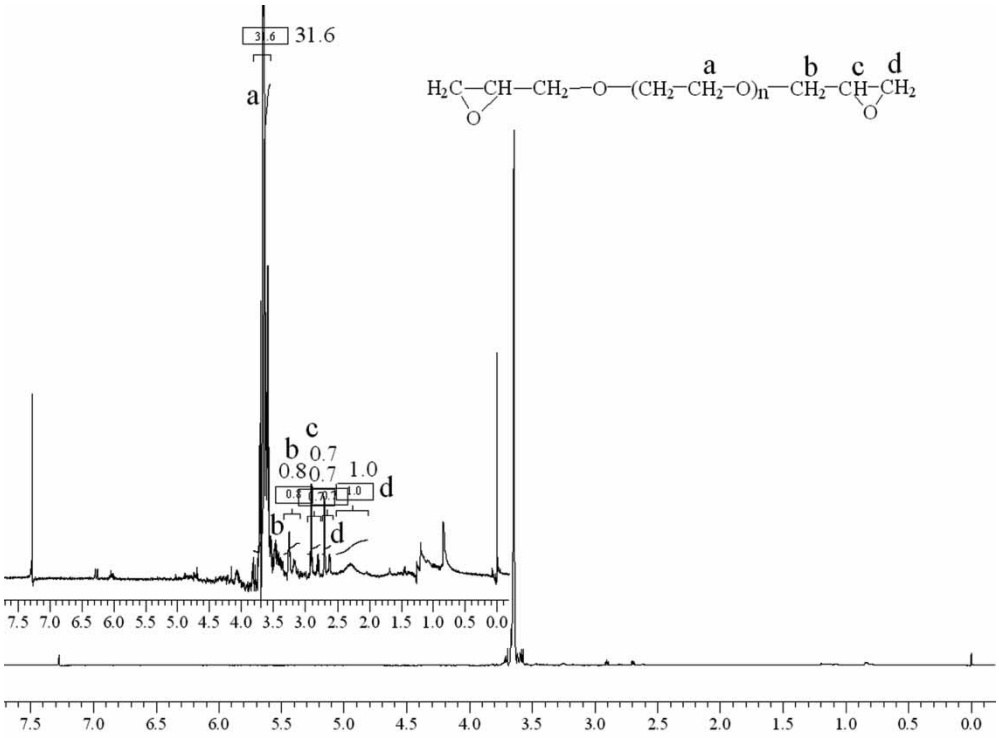

Fig. 1. The ^{1}H-NMR spectrum of poly(ethylene glycol)bisglycidyl ether.

From Figure 2, the resonance at 2.0 ppm corresponds to the terminal hydroxyl groups is present, but the resonances of methylene and methine protons of epoxy groups did not appear. Therefore, the epoxy groups of poly(ethylene glycol)bisglycidyl ether were hydrolyzed completely, they were all converted to hydroxyl groups, the hydroxyl functionality of tetrahydroxyl poly(ethylene glycol) is 3.96 (converted from epoxy groups: $1.96 \times 2 = 3.92$, unreacted hydroxyl

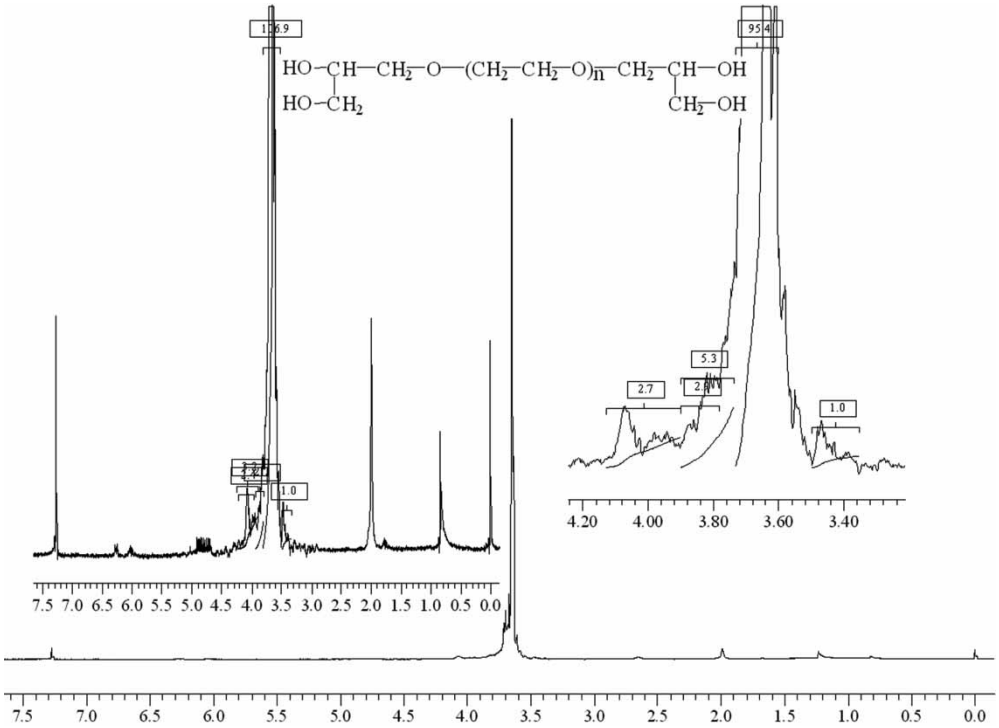

Fig. 2. The ^{1}H-NMR spectrum of tetrahydroxyl poly(ethylene glycol).

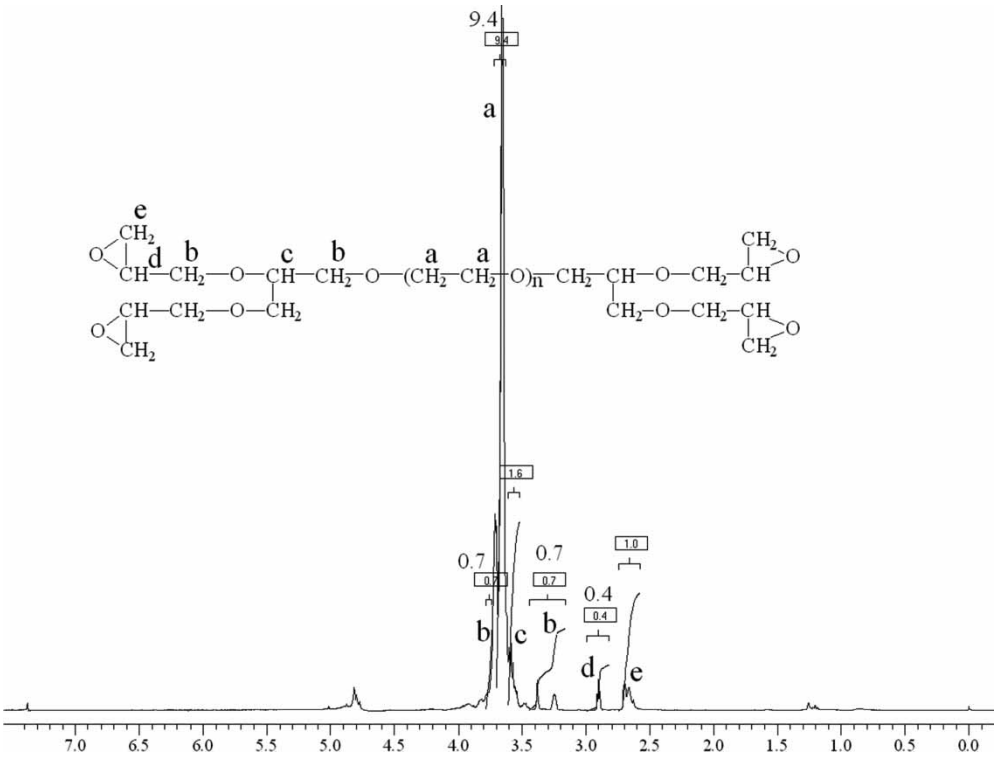

Fig. 3. The ^{1}H-NMR spectrum of poly(ethylene glycol) tetraglycidyl ether.

groups of PEG: 2−1.96 = 0.04, total: 3.92 + 0.04 = 3.96). The number-average molecular weight ($\bar{M}$n) of the tetrahydroxyl poly(ethylene glycol) was 1150 Da.

According to the above method, the theoretical intensity ratio of methylene (PEG, 3.6 ppm, 88) resonance to methine (glycidyl, 2.90 ppm, 3.96) resonance of poly(ethylene glycol) tetraglycidyl ether on ^{1}H-NMR spectrum is 22.2/1. From Figure 3, the real intensity ratio is 9.4/0.4 (23.5/1), therefore, the epoxide number of poly(ethylene glycol) bisglycidyl ether is 3.74, the real hydroxyl functionality of octahydroxyl poly(ethylene glycol) is 7.70 (converted from epoxy groups: 3.74 × 2 = 7.48, unreacted hydroxyl groups of tetrahydroxyl PEG: 3.96−3.74 = 0.22, total: 7.48 + 0.22 = 7.70). $\bar{M}$n of the octahydroxyl poly(ethylene glycol) was 1430 Da. The ^{1}H-NMR spectrum of octahydroxyl poly(ethylene glycol) was like that of tetrahydroxyl poly(ethylene glycol), so the figure is not shown here.

3.2 NMR Characterization of PEG-Supported PLGA Barbell-like Copolymers

The chemical structures of the resulting products were confirmed from both ^{1}H-NMR and ^{13}C-NMR spectra as shown in Figures 4 and 5.

From the ^{1}H and ^{13}C-NMR spectra of the synthesized PEG-supported PLGA barbell-like copolymers, it can be concluded that the $SnCl_2$ catalyzed direct melt polycondensation of lactic acid, glycolic acid and multi-hydroxyl end-group PEG was successful in producing PEG-supported PLGA barbell-like copolymers. In the ^{1}H-NMR spectra, the four major resonances corresponding to the methyl, methylene and methine groups of the poly(lactic acid), poly(glycolic acid) and PEG segments were observed at 1.4∼1.7 ppm (methyl of PLA), 3.5∼3.7 ppm (methylene of PEG), 4.6∼4.9 ppm (methylene of PGA) and 5.2 ppm (methine of

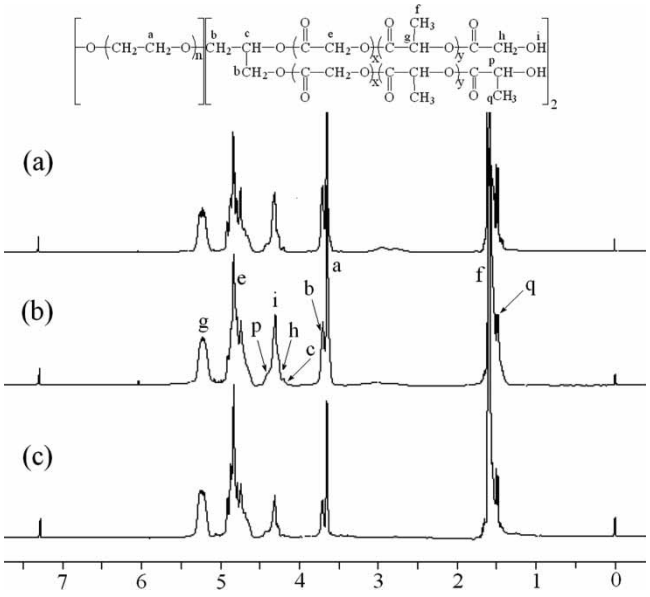

Fig. 4. ^{1}H-NMR spectra of PEG-supported PLGA barbell-like copolymers (a) triblock copolymer, (b) four arms copolymer, and (c) eight arms copolymer.

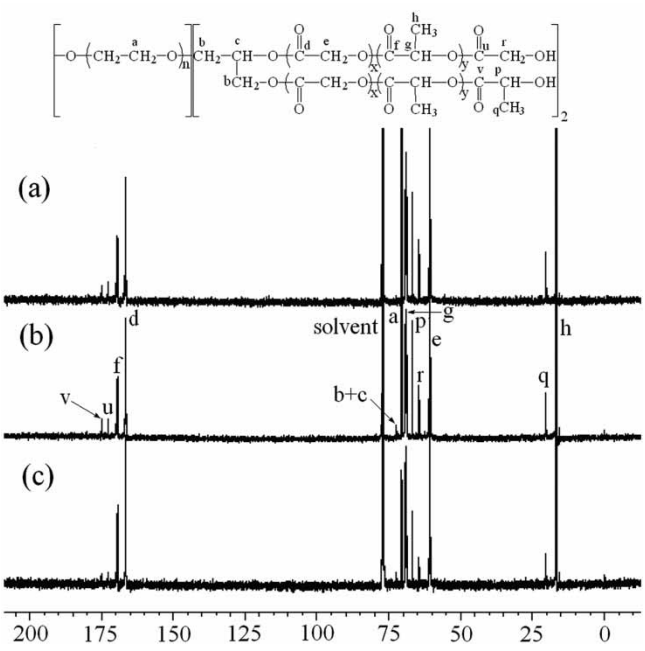

Fig. 5. ^{13}C-NMR spectra of PEG-supported PLGA barbell-like copolymers (a) triblock copolymer, (b) four arms copolymer, and (c) eight arms copolymer.

PLA). The resonance at 4.2∼4.4 ppm corresponds to the terminal methylene and methine of PGA and PLA. All these peaks were quite distinguishable in ^{1}H-NMR spectra of 2, 4 and 8 arms PEG-supported PLGA barbell-like copolymers. However, the methylene and methine protons of the glycerol ether units most likely overlaps with the large PEG methylene resonance. Also, in the ^{13}C-NMR spectra, the seven major resonances corresponding to the carbonyl, methyl, methylene and methine groups of the poly(lactic acid), poly(glycolic acid) and PEG segments were observed at 16.5 ppm (methyl of PLA), 60.2 ppm (methylene of PGA), 69.0 ppm (methine of PLA), 70.4 ppm (methylene of PEG), 166 ppm (carbonyl of PGA) and 169 ppm (carbonyl

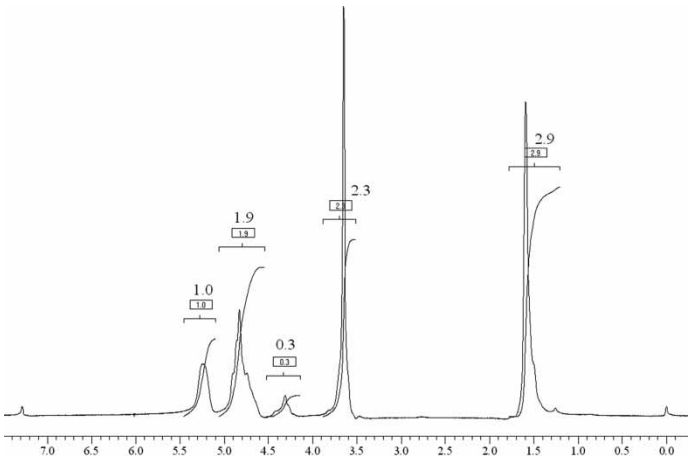

Fig. 6. The ^{1}H-NMR spectrum of $(PLGA)_2$-PEG-$(PLGA)_2$ barbell-like copolymers (S2).

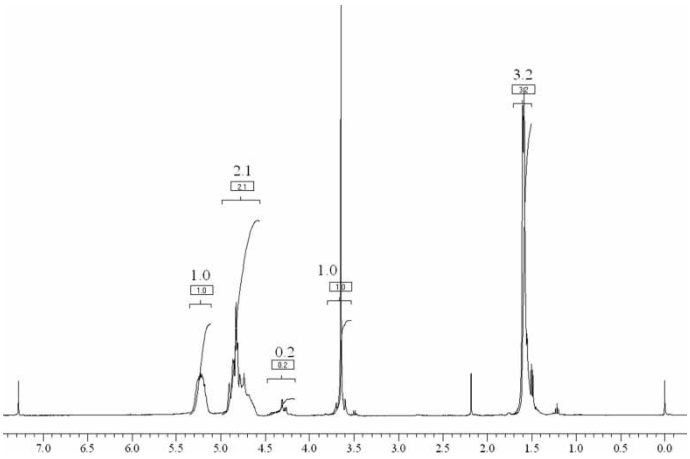

Fig. 7. The ^{1}H-NMR spectrum of $(PLGA)_4$-PEG-$(PLGA)_4$ barbell-like copolymers (S3).

of PLA). The resonance at 174 ppm and 172 ppm correspond to the terminal carbonyls of PLA and PGA, respectively.

3.3 Molecular Weight and Arm Length of Barbell-like Copolymers

From the above-mentioned analysis, the methylenes of tetrahydroxyl PEG have responded at 3.5∼3.9 ppm, so one molar tetrahydroxyl PEG contained 96 ($88 + 4 \times 1.96 = 96$) mol methylene protons. From Figure 6, the real intensity ratio of methylene (tetrahydroxyl PEG, 3.6 ppm) resonance to methine (LA, 5.25 ppm) is 2.3/1.0 (=96/41.5), the real intensity ratio of methylene (tetrahydroxyl PEG, 3.6 ppm) resonance to methylene (GA, 4.6∼5.0 ppm) is 2.3/1.9 (96/78.8). Furthermore, the peak at 4.25∼4.45 ppm is the

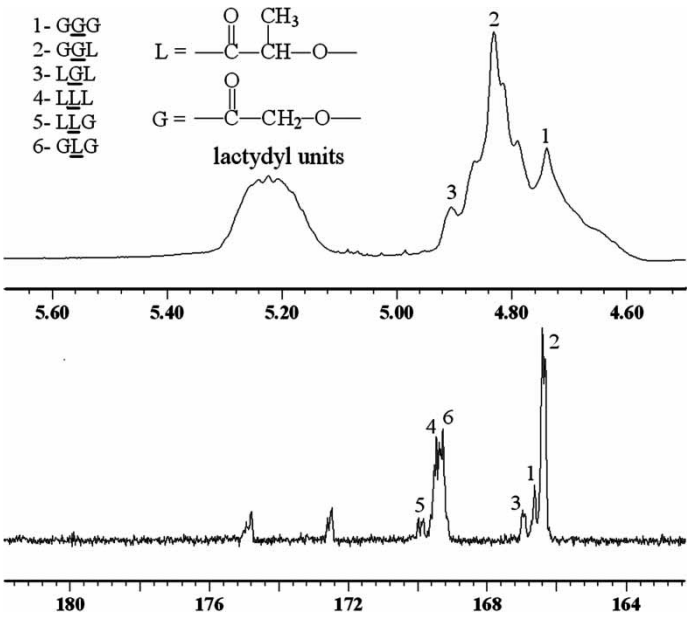

Fig. 8. The sequence analysis of PLGA in barbell-like copolymers (four arms).

Table 1. Molecular weight and molecular weight distributions of PEG-supported PLGA barbell-like copolymers determined by GPC and ^{1}H-NMR

Sample	$\bar{M}n_{Th}$[a] ($\times 10^4$ Da)	$\bar{M}n_{NMR}$[b] ($\times 10^4$ Da)	$\bar{M}n$[c] ($\times 10^4$ Da)	$\bar{M}w$[c] ($\times 10^4$ Da)	PDI[c]
S1	0.40	0.39	0.22	0.25	1.12
S2	0.69	0.67	0.33	0.43	1.33
S3	1.28	1.67	0.23	0.29	1.24

[a]Theoretic average molecular weight which were calculated with feed ratio of materials.
[b]Measured by ^{1}H-NMR analysis.
[c]Determined by GPC in THF.

terminal methine (LA) and methylene (GA) (besides terminal hydroxyl) resonance, in other words, one molar $(PLGA)_2$-PEG-$(PLGA)_2$ copolymer contained 3.96 mol LA and GA terminal groups, the molar ratio of the terminal LA group to GA group is about 1/1. Therefore, one molar $(PLGA)_2$-PEG-$(PLGA)_2$ on average contained 43.5 mol LA and 41.4 mol GA monomer units. According to this result, $\bar{M}n$ of the $(PLGA)_2$-PEG-$(PLGA)_2$ copolymer was about 6700 Da ($43.5 \times 72 + 41.4 \times 58 + 1150 = 6700$), the molar ratio of LA units to GA units is 1.05, every PLGA arm on average contained ten LA units, eleven GA units and one terminal unit, total monomer units is 22.

Using the same analytical methods for $(PLGA)_2$-PEG-$(PLGA)_2$ copolymer, one molar octahydroxyl PEG contained 111 ($96 + 4 \times 3.74 = 111$) mol methylene protons. From Figure 7, the real intensity ratio of methylene (tetrahydroxyl PEG, 3.6 ppm) resonance to methine (LA, 5.25 ppm) is 1.0/1.0 (111 mol LA units), the real intensity ratio of methylene (tetrahydroxyl PEG, 3.6 ppm) resonance to methylene (GA, 4.6∼5.0 ppm) is 1.0/2.1 (117 mol GA units). Furthermore, the 7.70 mol terminal monomer units should be added. Therefore, one molar $(PLGA)_4$-PEG-$(PLGA)_4$ on average contained 114.8 mol LA and 120.8 mol GA monomer units. According to these results, $\bar{M}n$ of $(PLGA)_4$-PEG-$(PLGA)_4$ copolymer was about 16700 Da, the molar ratio of LA units to GA units was 0.95, every PLGA arm on average contained fourteen LA units, fifteen GA units and one terminal unit, with total monomer units equal to 30.

The polycondensation system of PLGA involves two reaction equilibrium: dehydration equilibrium for esterification and ring-chain equilibrium involving the depolymerization of PLGA into lactide, glicolide and DL-3-methyl glycolide (25, 26). Figure 8 shows the sequence analysis for PLGA in the four arms barbell-like copolymers. In accord with the literature (27), we note that the -GLG-, -LLL-, and -GGL-type sequences are more than others. It should originate from ring-opening polymerization of cyclic monomers.

The results of molecular weight determinations by both by NMR and GPC are shown in Table 1. Although the measured molecular weights of dendritic and star copolymers by GPC are lower than the actual data (because of lower hydrodynamic volume of these types of compounds than linear analogs) (28, 29), the GPC analysis is a reliable method for determining the molecular weight distributions of the prepared copolymers.

GPC showed that the molecular weight distributions of the barbell-like copolymers were narrow, and $\bar{M}n$ $\bar{M}w$ were all smaller than $\bar{M}n_{Th}$. The values given by GPC are affected not only by the size of the molecule but also the structure of the molecule. For the tri-block copolymer (S1), the value of $\bar{M}n_{GPC}$ is a little smaller than those of $\bar{M}n_{Th}$ and $\bar{M}n_{NMR}$, but for eight-arm copolymers (S3), the values of $\bar{M}n_{GPC}$ is differ from those of $\bar{M}n_{Th}$ and $\bar{M}n_{NMR}$. The $\bar{M}n_{NMR}$'s values accord with the theoretical values closely.

4. Conclusions

The hydroxyl functionality of tetra- and octa-hydroxyl PEG was 3.96 and 7.70, respectively. The number-average molecular weight of $(PLGA)_2$-PEG-$(PLGA)_2$ and $(PLGA)_4$-PEG-$(PLGA)_4$ barbell-like copolymers determined by ^{1}H-NMR analysis was 6700 and 16700 Da, respectively. Compared with the theoretical values, the number-average molecular weights of $(PLGA)_n$-PEG-$(PLGA)_n$ barbell-like copolymers determined by ^{1}H-NMR analysis gave reasonably consistent values, but the values determined by gel permeation chromatography (GPC) were less than the theoretical values. All these results indicated that $(PLGA)_n$-PEG-$(PLGA)_n$ copolymers have a linear-dendritic structures.

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