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Synthesis and characterization of biodegradable and biocompatible amphiphilic block copolymers bearing pendant amino acid residues

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ABSTRACT

Polylactide (PLA)-based biodegradable and biocompatible amphiphilic block copolymers bearing pendant amino acid residues were synthesized through a relatively easy and efficient way. The composition and structure of these copolymers were characterized by gel permeation chromatography (GPC) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy. The self-assembly behavior of the copolymers was investigated by fluorescence (FL), dynamic light scattering (DLS), and transmission electron microscope (TEM). It was shown that aggregates less than 100 nm in average size were formed by these copolymers, which changed from micelles to vesicles with the variation of the block length. In addition, the in vitro cytotoxicity of these copolymers was determined and compared with that of PEO-*b*-PLA in the presence of Bel-7402 cells. The result suggested that the block copolymers PAGE/cys-*b*-PLA exhibited better biocompatibility. Therefore, these PLA-based copolymers are expected to find promising applications in drug delivery or tissue engineering.

1. Introduction

Amphiphilic block copolymers can self-assemble into nanosized aggregates of various morphologies in aqueous solution, such as micelles, cylinders, or vesicles [1–6]. These supramolecular assemblies hold great potential applications in drug delivery, diagnostic biosensors and tissue engineering [7–11]. Therefore, it has been a hot topic for a long time to design diverse biocompatible and biodegradable amphiphilic block copolymers.

Polylactide (PLA), an important kind of synthetic polymer, has been widely used in various biomedical applications due to its biodegradability, good biocompatibility and excellent shaping and modeling properties [12–15]. A number of PLA-based amphiphilic block copolymers have been investigated in terms of various of biomedical applications. In many cases, poly(ethylene glycol) (PEG) was chosen as hydrophilic block because of its nontoxicity, hydrophilicity and biocompatibility [16–27]. Nevertheless, this kind of copolymers of PLA and PEG were not functionalized except at chain end. This feature may limit their use in some fields. Recently, there has been an increasing interest in the synthesis of PLA-based amphiphilic block copolymers bearing functional groups in the side chains. Several research groups have synthesized PLA-based amphiphilic block copolymers bearing hydroxyl, carboxyl or amine functional groups in the side chains [28–35]. The presence of pendant functional groups helps to enhance affinity of the polymers to cells or proteins and provides possibilities for further functionalization. However, in these specific cases the pendant functional groups must be protected during polymerization, and then the protecting groups have to be removed from the polymer, which may destroy the sensitive polyester segment. An easy and efficient strategy is high desirable to prepare PLA-based functional biodegradable amphiphilic block copolymers.

Poly(allyl glycidyl ether) (PAGE), a functional analog of PEG, has been of great interest during the past years due to its high functionality and to the fact that PAGE with a relatively narrow molecular weight distribution is accessible by anionic polymerization [36]. The double bonds along the polymer chain can react with ω-functional mercaptons via free-radical addition in quantitative yields under mild conditions [37]. Moreover, this reaction can tolerate the presence of most functional groups (–OH, –NH_2, –COOH, etc.) [38]. If PAGE is used to copolymerize with LA, various kinds of functional amphiphilic block copolymers based on PLA can be obtained from the copolymer intermediates poly(allyl glycidyl ether)-*b*-polylactide (PAGE-*b*-PLA) without the resource to protecting groups chemistry. This approach is an easy and efficient route to PLA-based functional amphiphilic block copolymers. To the best of our knowledge, there was no reports on the block copolymer PAGE-*b*-PLA.

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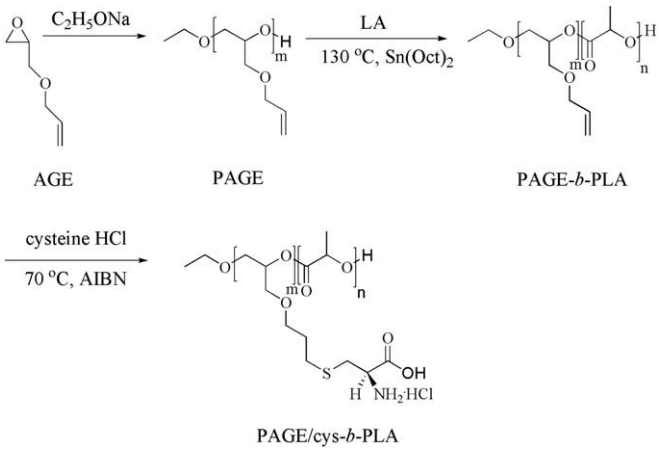

Scheme 1. Synthesis of PAGE/cys-*b*-PLA copolymers.

It was well-known that amino acids are widely distributed in living organisms as important fragments. The advantage of using amino acids to modify polymers is that amino acid residues might modulate the bio-availability of polymers. In this work, a series of PLA-based amphiphilic block copolymers bearing amino acid residues were designed and synthesized as shown in Scheme 1. We synthesized the copolymer intermediates PAGE-*b*-PLA first, then cysteine hydrochloride molecules were conjugated to the pendant double bonds of PAGE-*b*-PLA via free-radical addition to give the amphiphilic block copolymers poly(allyl glycidyl ether/cysteine HCl)-*b*-polylactide (PAGE/cys-*b*-PLA). The in vitro cytotoxicity of the copolymers PAGE/cys-*b*-PLA was determined and compared with that of PEO-*b*-PLA. The result proved that the copolymers PAGE/cys-*b*-PLA exhibited better compatibility. The composition and structure of the copolymers PAGE/cys-*b*-PLA were characterized, and their self-assembly behavior was investigated in aqueous solution. It was found that these copolymers can self-assemble into micelles or vesicles (less than 100 nm in average size) by adjusting the block length.

2. Experimental section

2.1. Materials

Allyl glycidyl ether (AGE) was dried over CaH_2 for 24 h at room temperature and distilled under reduced pressure. Tin(II) bis(2-ethylhexanoate) was purchased from Alfa Aesar and was used as received. DL-Lactide was obtained from Glaco Ltd and used without further purification. L-Cysteine hydrochloride was obtained from Beijing Biodee Biotechnology Ltd. *N*,*N*-Dimethylformamide (DMF) was dried by refluxing over CaH_2 and distilled under reduced pressure before use. 2,2′-Azobisisobutyronitrile (AIBN) from Aldrich was recrystallized twice from ethanol. Dialysis tubing (molecular weight cutoff 2000 Da) was purchased from Shanghai Green Bird Technology Development Ltd. Other reagents and solvents used were of analysis grade and were dried according to the conventional methods.

2.2. Measurements

^{1}H NMR spectra were measured on a Bruker-400 NMR instrument using $CDCl_3$, DMSO-d_6 and D_2O–DMSO-d_6 (6:1 v/v) as solvents. Molecular weight and molecular weight distribution of the polymer samples were determined by gel permeation chromatography (GPC) using a LC-10AVP apparatus equipped with three columns (SHIMADZU Shim-pack GPC-803) in series (eluent, DMF; flow rate, 1.0 mL/min). The GPC chromatogram was calibrated against standard polystyrene samples at 40 °C. Fluorescence spectra were recorded on a CARY Eclipse FL spectrometer in a right-angle geometry (90° collecting optics) at 25 °C. Hydrodynamic diameter of the micelles was measured by using Malvern Zetasizer nano-ZS-90. Transmission electron microscope (TEM) images were obtained using a TeenaiG220 S-TWIN operating at an acceleration voltage of 200 kV. The dialyzed solutions of the copolymers PAGE/cys-*b*-PLA (0.4 mg/mL) were used for TEM detection. A drop of the solution was placed onto TEM copper/carbon grid and the excess solution was blotted up using a strip of filter paper, then the sample was allowed to dry at room temperature before observation.

2.3. Synthesis of poly(allyl glycidyl ether) (PAGE) homopolymers

A typical example for the polymerization reaction is described as following. Under nitrogen, 0.50 g (7.38 mmol) of freshly prepared C_2H_5ONa and 2 mL of anhydrous xylene were introduced into a flame-dried and nitrogen-purged Schlenk tube equipped with a stirrer, followed by sonication for 15 min. When C_2H_5ONa was scattered evenly into xylene, 15.26 mL (0.13 mol) of AGE was injected using a glass syringe. The reaction mixture was heated at 100 °C for 24 h. After that, 0.42 mL (7.38 mmol) of acetic acid was added to stop the polymerization reaction, and then the system was cooled to room temperature.

The crude PAGE was dissolved in CH_2Cl_2, dried with Na_2CO_3 overnight and then filtered. The filtrate was concentrated under reduced pressure, and the solution was precipitated with hexane to give a light yellow viscous liquid. The results are summarized in Table 1.

$PAGE_{2k}$: ^{1}H NMR, δ ($CDCl_3$, ppm): 1.21 (t, 3H, C***H***$_3$CH$_2$O–), 3.47–3.67 (m, 80H, –C***H***$_2$C***H***(C***H***$_2$O–)O–), 4.01 (d, 32H, –OC***H***$_2$CH=CH$_2$), 5.20 (d, 16H, trans, –CH=C***H***$_2$), 5.28 (d, 16H, *cis*, –CH=C***H***$_2$), 5.91 (m, 16H, –C***H***=CH$_2$). GPC: $M_w = 8750$, $M_w/M_n = 1.05$.

Table 1
Preparation and characteristics of the PAGE homopolymers and PAGE-*b*-PLA copolymers, I = initiator, M = monomer.

Sample	Initiator (g)	Monomer (g)	Molar ratio (I/M)	M_n[a]	M_n[b]	M_w[c]	M_w/M_n[c]
$PAGE_{2k}$	0.50	14.46	1/17.5	2000	1820	8750	1.05
$PAGE_{4k}$	0.40	23.70	1/35.0	4000	3800	10 130	1.03
$PAGE_{2k}$-*b*-PLA_{2k}	4.30	4.57	1/13.5	4000	4200	11 690	1.56
$PAGE_{2k}$-*b*-PLA_{4k}	2.67	5.36	1/25.3	6000	5480	12 430	1.90
$PAGE_{4k}$-*b*-PLA_{4k}	4.22	4.43	1/28.0	8000	7470	13 050	1.63
$PAGE_{4k}$-*b*-PLA_{8k}	2.22	4.72	1/56.1	12 000	11 500	15 100	1.51

[a] Calculated from the molar ratio of monomer and initiator.
[b] Determined by ^{1}H NMR in $CDCl_3$.
[c] Measured by GPC in DMF with polystyrene as calibration standard.

2.4. Synthesis of poly(allyl glycidyl ether)-b-polylactide (PAGE-b-PLA)copolymers

PAGE-*b*-PLA copolymers were prepared by ring-opening polymerization of LA with PAGE in the presence of $Sn(Oct)_2$ as catalyst. 4.72 g of LA (32.81 mmol), 2.22 g of $PAGE_{4k}$ (0.58 mmol) and 0.34 mL of $Sn(Oct)_2$ in xylene (1.7×10^{-1} mol/mL) were charged into a flame-dried and nitrogen-purged Schlenk tube with a stirrer. The tube was purged with nitrogen and degassed several times. Then, the flask was sealed under vacuum and put into a pre-heated oil bath. The reaction was carried out at 130 °C for 20 h, it was then stopped by removing the flask from the oil bath. Purification was performed by dissolving the reaction mixture in a small amount of CH_2Cl_2 and pouring it into an excess of hexane with stirring. The copolymers were collected and dried in vacuum. The results are also summarized in Table 1.

$PAGE_{2k}$-*b*-PLA_{2k}: ^{1}H NMR, δ ($CDCl_3$, ppm): 1.21 (t, 3H, C***H***$_3$CH$_2$O–), 1.54 (m, 96H, –COCH(C***H***$_3$)O–), 3.47–3.65 (m, 80H, –C***H***$_2$C***H***(C***H***$_2$O–)O–), 4.00 (d, 32H, –C***H***$_2$CH=CH$_2$), 5.15–5.29 (m, 64H, –CH=C***H***$_2$, –COC***H***(CH$_3$)O–), 5.90 (m, 16H, –C***H***=CH$_2$). GPC: $M_w = 11\,690$, $M_w/M_n = 1.56$.

2.5. Synthesis of poly(allyl glycidyl ether/cysteine HCl)-b-polylactide (PAGE/cys-b-PLA) amphiphilic block copolymers

Amphiphilic block copolymers PAGE/cys-*b*-PLA were prepared by free-radical addition of cysteine hydrochloride onto PAGE-*b*-PLA copolymers. In a typical experiment, the solution containing 1.50 g (4.60 mmol C=C) of $PAGE_{4k}$-*b*-PLA_{8k}, 8.07 g (45.96 mmol) of cysteine HCl and 0.45 g (2.76 mmol) of AIBN (molar ratio: [C=C]/[SH]/[AIBN] = 1:10:0.6) in 60 mL of anhydrous DMF was degassed for 1 h under nitrogen flow, then the mixture was heated at 70 °C for 24 h. After that, the mixture was cooled to room temperature and DMF was evaporated under reduced pressure. The resultant products were dialyzed against methanol using a dialysis membrane tube (M_w cutoff 2000 Da) at room temperature for 4 days to remove the excess of cysteine. At last, methanol in the dialyzed solution was evaporated under reduced pressure and dried in vacuum. The product was obtained as light yellow powder.

$PAGE_{2k}$/cys-*b*-PLA_{2k}: ^{1}H NMR, δ (DMSO-d_6, ppm): 3.23–3.58 (m, 28H, –C***H***$_2$C***H*** (C***H***$_2$OC***H***$_2$–)O–), 1.43 (m, 12H, –COCH(C***H***$_3$)O–), 5.15 (m, 4H, –COC***H***(CH$_3$)O–), 1.71 (t, 3H, –OCH$_2$C***H***$_2$CH$_2$S–), 2.54 (t, 3H, –OCH$_2$CH$_2$C***H***$_2$S–), 2.74–2.96 (m, 3H, –SC***H***$_2$CH(NH)COOH), 3.68, 4.54 (d, 1.5H, –SCH$_2$C***H***(NH)COOH).

2.6. Preparation of the self-assembly aggregates of the copolymers PAGE/cys-b-PLA in water

In this study, the dialysis method was employed to prepare the self-assembly aggregates of the amphiphilic block copolymers PAGE/cys-*b*-PLA. 40 mg of the copolymer was dissolved in 5 mL of DMF in a 50-mL round-bottom flask with a stirrer, and 40 mL of the twice-distilled water was added dropwise (ca. one drop/15 s). Then, the solution was dialyzed against the twice-distilled water using a dialysis membrane tube (M_w cutoff 2000 Da) for 3 days to remove DMF. The polymer solution and twice-distilled water were added to a 100-mL volumetric flask to make the polymer solution of 0.4 mg/mL. The samples were sonicated to homogenize the solution.

2.7. Cytotoxicity of the amphiphilic block copolymers PAGE/cys-b-PLA

Human hepatocellular carcinoma (Bel-7402 cells) was used as the model to study the cytotoxicity of $PAGE_{2k}$/cys-*b*-PLA_{4k}. The Bel-7402 cells were routinely cultured in tissue culture flasks with RPMI 1640 medium, containing 10% fetal bovine serum and incubated at 37 °C in a humidified atmosphere with 95% air and 5% CO_2. The culture medium was refreshed every two days. When the cells became almost confluent after 5 days, they were released by treatment with 0.25% trypsin. Then the cells were counted to 10^4 cells/cm^2 and 200 μl of the cells suspension were pipetted into 96-well tissue culture plate. After 4 h of culture, the medium was replaced with RPMI 1640 medium containing 0.05–0.4 mg/mL of $PAGE_{2k}$/cys-*b*-PLA_{4k}, and continued to culture another 48 h. The same experiments were carried out for PEO_{2k}-*b*-PLA_{4k}. Cells proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay based on succinic

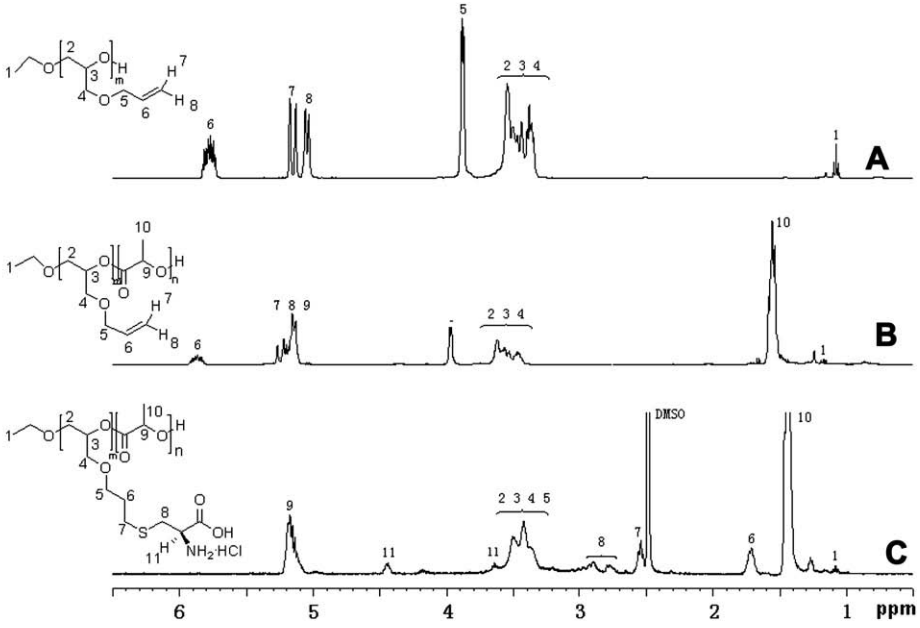

Fig. 1. ^{1}H NMR of (A) $PAGE_{2k}$ in $CDCl_3$, (B) $PAGE_{2k}$-*b*-PLA_{4k} in $CDCl_3$, and (C) P(AGE_{2k}/cys)-*b*-PLA_{4k} in DMSO-d_6.

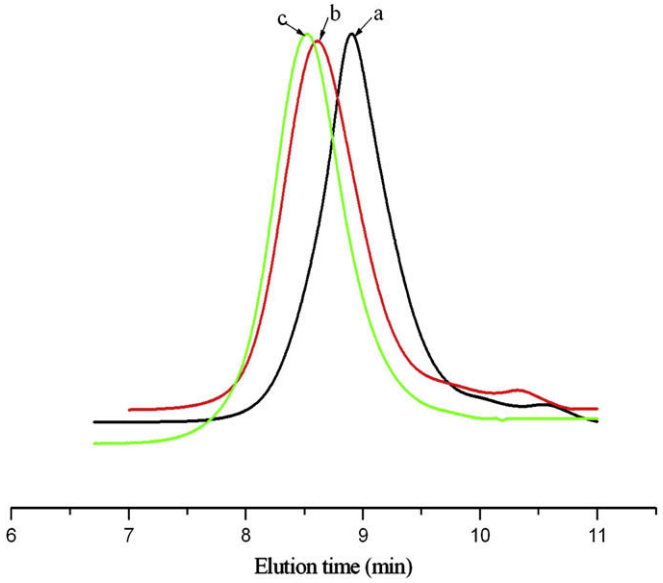

Fig. 2. GPC traces of (a) $PAGE_{2k}$, (b) $PAGE_{2k}$-*b*-PLA_{2k} and (c) $PAGE_{2k}$-*b*-PLA_{4k}.

dehydrogenase activity at OD_{490nm} ($n = 6$), 630 nm was chosen as the reference wavelength.

3. Results and discussion

3.1. Synthesis of amphiphilic block copolymers bearing amino acid residues

The amphiphilic block copolymers PAGE/cys-*b*-PLA were synthesized according to the procedure shown in Scheme 1. The copolymers PAGE-*b*-PLA were synthesized first by ring-opening polymerization of LA with PAGE as macroinitiator and $Sn(Oct)_2$ as catalyst. The double bonds in the side chains provided the possibility to react with ω-functional mercaptons via free-radical addition. The amphiphilic block copolymers bearing amino acid residues were obtained by the free-radical addition of cysteine hydrochloride onto the double bonds of PAGE-*b*-PLA.

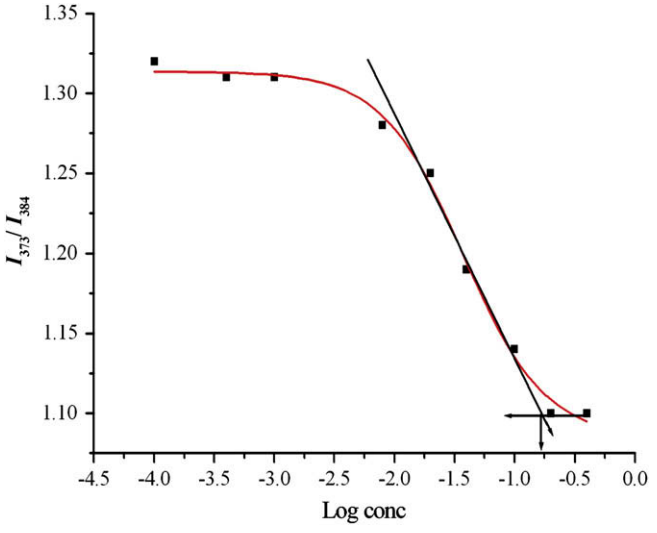

Fig. 3. Plot of the ratio I_{373}/I_{384} (from pyrene excitation spectra at $\lambda_{exc} = 335$ nm) versus the logarithm of the P(AGE_{2k}/cys)-*b*-PLA_{2k} concentration.

PAGE monoethyl ethers were obtained by anionic ring-opening polymerization of AGE using C_2H_5ONa as initiator. Fig. 1A showed the ^1H NMR spectrum of $PAGE_{2k}$. The degree of polymerization of PAGE was calculated from the relative integration intensities of protons of the methyl end group (–C***H***$_3$) at 1.21 ppm and protons of the pendant double bonds (–C***H***=CH_2) at 5.91 ppm. M_n determined from ^1H NMR spectra was close to the theoretical value. It can be seen that the GPC trace of $PAGE_{2k}$ was narrow and monomodal (Fig. 2a), which indicated that PAGE with narrow molecular weight distribution was obtained.

The block copolymer intermediates PAGE-*b*-PLA were prepared by ring-opening polymerization of LA using PAGE as macroinitiator in the presence of $Sn(Oct)_2$. The length of PLA blocks was controlled by the molecular ratio of the monomer to the initiator. In this way, a series of block copolymers differing in the length of the polymer blocks were obtained as listed in Table 1. Fig. 1B showed the ^1H NMR spectrum of $PAGE_{2k}$-*b*-PLA_{4k}. Compared to the spectrum of $PAGE_{2k}$, the new peak at 1.54 ppm is attributed to the C***H***$_3$ of lactic acid. The peaks at 5.16–5.29 ppm are attributed to the C***H*** of lactic acid and the –CH=C***H***$_2$ of AGE. The peaks at 3.47–3.65, 4.00 and 5.90 ppm are attributed to –C***H***$_2$–C***H***($C***H***_2$O–)O–, –C***H***$_2$–CH=CH_2 and –C***H***=CH_2 of PAGE segment, respectively. The actual molar ratio of the two components of the copolymers was estimated by comparing the integrations of PLA methyl proton signals at 1.54 ppm and signals at 5.86 ppm assigned to the double bonds of the PAGE blocks. Compared to Fig. 2a, the GPC traces of the copolymers $PAGE_{2k}$-*b*-PLA_{2k} and $PAGE_{2k}$-*b*-PLA_{4k} (Fig. 2b, c) showed narrow and monomodal peaks and shifted to the higher molecular weight region. These results indicated that the copolymerization of PAGE and LA proceeded successfully.

Finally, the amino acid molecules were conjugated to the pendant double bonds of the copolymers PAGE-*b*-PLA via free-radical addition reaction to give the PLA-based functional amphiphilic block copolymers. In the ^1H NMR spectrum of the resulted copolymer of $PAGE_{2k}$/cys-*b*-PLA_{4k} (Fig. 1C), the signals of cysteine HCl residues at 2.74–2.90 ppm (–S–C***H***$_2$–CH(NH_2)COOH), 3.68 and 4.54 ppm (–S–CH_2 –C***H***(NH_2)COOH) and the signals at 1.71 ppm (–CH_2C***H***$_2CH_2$S–) and 2.54 ppm (–CH_2CH_2C***H***$_2$S–) were observed clearly. At the same time, the signal at 5.84 ppm (–C***H***=CH_2), which is the characteristic of the pendant double bonds of PAGE block, disappeared completely, indicating that the occurrence of the free-radical addition between the double bonds of PAGE-*b*-PLA and –SH groups of cysteine hydrochloride.

3.2. Self-assembly properties of the amphiphilic block copolymers PAGE/cys-b-PLA

The self-assembly behavior of the amphiphilic block copolymers PAGE/cys-*b*-PLA in aqueous solution was investigated by fluorescence technique using pyrene as probe. Fig. 3 showed a plot of the pyrene fluorescence intensity ratio I_{373}/I_{384} (I_{373}, the first peak on the excitation spectra; I_{384}, the third peak) versus the logarithm of the $PAGE_{2k}$/cys-*b*-PLA_{2k} concentration. It can be seen that the ratio

Table 2
Characteristics of the self-assembly aggregates of PAGE/cys-*b*-PLA copolymers.

Copolymers	D_h[a] (nm)	PDI[a]	cac[b] (mg/mL)
P(AGE_{2k}/cys)-*b*-PLA_{2k}	23	0.28	0.16
P(AGE_{2k}/cys)-*b*-PLA_{4k}	32	0.25	0.09
P(AGE_{4k}/cys)-*b*-PLA_{4k}	39	0.26	0.11
P(AGE_{4k}/cys)-*b*-PLA_{8k}	43	0.27	0.06

[a] Mean hydrodynamic diameters (D_h) and polydispersity index (PDI) of the copolymers determined at 0.4 mg/mL by dynamic light scattering at 25 °C.
[b] The critical aggregate concentration (cac) determined in water at 25 °C by fluorescence technique using pyrene as probe.

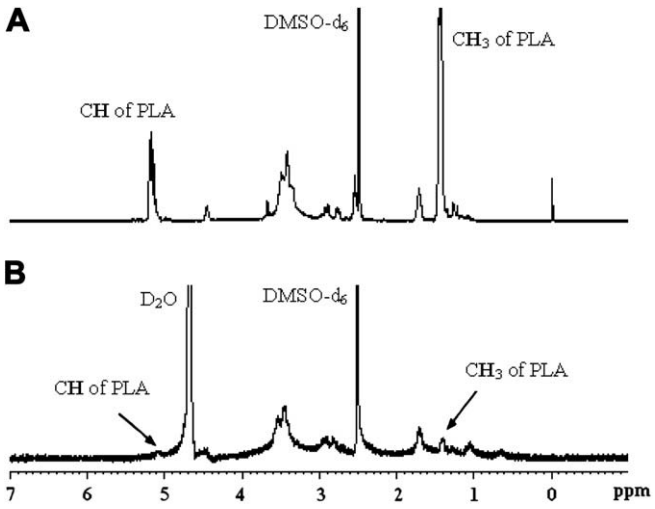

Fig. 4. ^{1}H NMR of PAGE$_{4k}$/cys-*b*-PLA$_{8k}$: (A) in DMSO-d_6, and (B) in D_2O–DMSO-d_6 (6:1, v/v) mixed solvent.

was almost constant at low polymer concentration. However, from a certain concentration, it started to decrease steadily with increasing concentration of the copolymer and finally reached a plateau. This phenomenon resulted from the transfer of pyrene molecules from a water environment to the hydrophobic one, indicating the formation of self-assembled aggregates. The critical aggregate concentration (cac) is an important parameter to describe the thermodynamic stability of self-assembled aggregates in aqueous solution. The cac value was determined from the intersection of the two tangent lines as shown in Fig. 3, and the result is summarized in Table 2. The formation of self-assembly aggregates was further confirmed by ^{1}H NMR (Fig. 4). The signals of methyl protons at 1.5 ppm and methenal protons at 5.1 ppm of the hydrophobic PLA segments can be seen clearly in DMSO-d_6 (Fig. 4A). However, these signals became very weak in the mixed solvent D_2O–DMSO-d_6 (6:1 v/v) (Fig. 4B). This observation suggested that the hydrophobic PLA segments were located within the hydrophobic region of the resulted self-assembly aggregates and its mobility was restricted. Additionally, the signals of the ethylene oxy units of the PAGE at 3.23–3.58 ppm and the signals of cysteine HCl residues at 2.74–2.96 ppm can be seen clearly in Fig. 4B. This means that the PAGE/cys segments of the copolymers exist near the shell in the resultant polymeric aggregates.

The morphologies of the self-assembly aggregates generated from the copolymers PAGE/cys-*b*-PLA were investigated using the technique of TEM. As shown in Fig. 5, the copolymers PAGE$_{2k}$/cys-*b*-PLA$_{2k}$ and PAGE$_{2k}$/cys-*b*-PLA$_{4k}$ formed spherical micellar aggregates. As the hydrophilic and hydrophobic block length increased within PAGE$_{4k}$/cys-*b*-PLA$_{4k}$ and PAGE$_{4k}$/cys-*b*-PLA$_{8k}$, aggregates with a vesicular morphology appeared (Fig. 5C and D). In all, the copolymers PAGE/cys-*b*-PLA can self-assemble into aggregates with micellar and vesicular morphologies by adjusting the block length. The dynamic light scattering analysis of the aggregates is shown in Table 2 and Fig. 6. At a concentration of 0.4 mg/mL in aqueous solution, the average diameter of the aggregates was in the range of 23–43 nm, which was nearly consistent with the TEM images. Moreover, all self-assembly aggregates had a narrow size distribution.

3.3. Biocompatibility of the copolymers PAGE/cys-b-PLA

In order to ensure biocompatibility of PAGE/cys-*b*-PLA, the in vitro cytotoxicity of the copolymer PAGE$_{2k}$/cys-*b*-PLA$_{4k}$ was

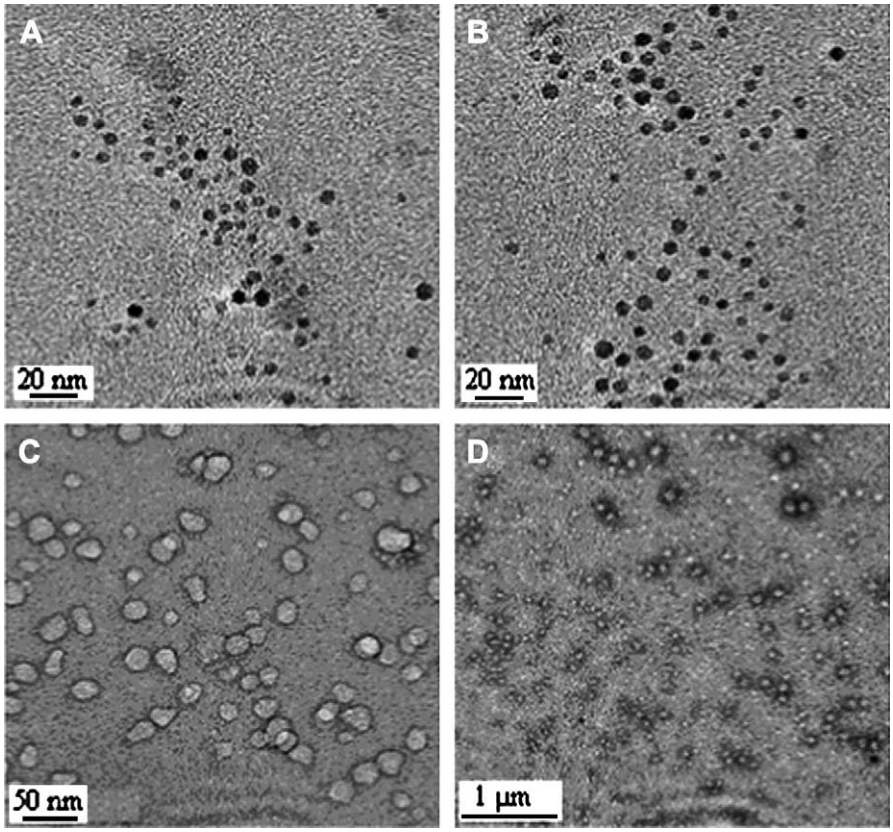

Fig. 5. TEM images of the self-assembled aggregates of PAGE/cys-*b*-PLA: (A) PAGE$_{2k}$/cys-*b*-PLA$_{2k}$, (B) PAGE$_{2k}$/cys-*b*-PLA$_{4k}$, (C) PAGE$_{4k}$/cys-*b*-PLA$_{4k}$ and (D) PAGE$_{4k}$/cys-*b*-PLA$_{8k}$.

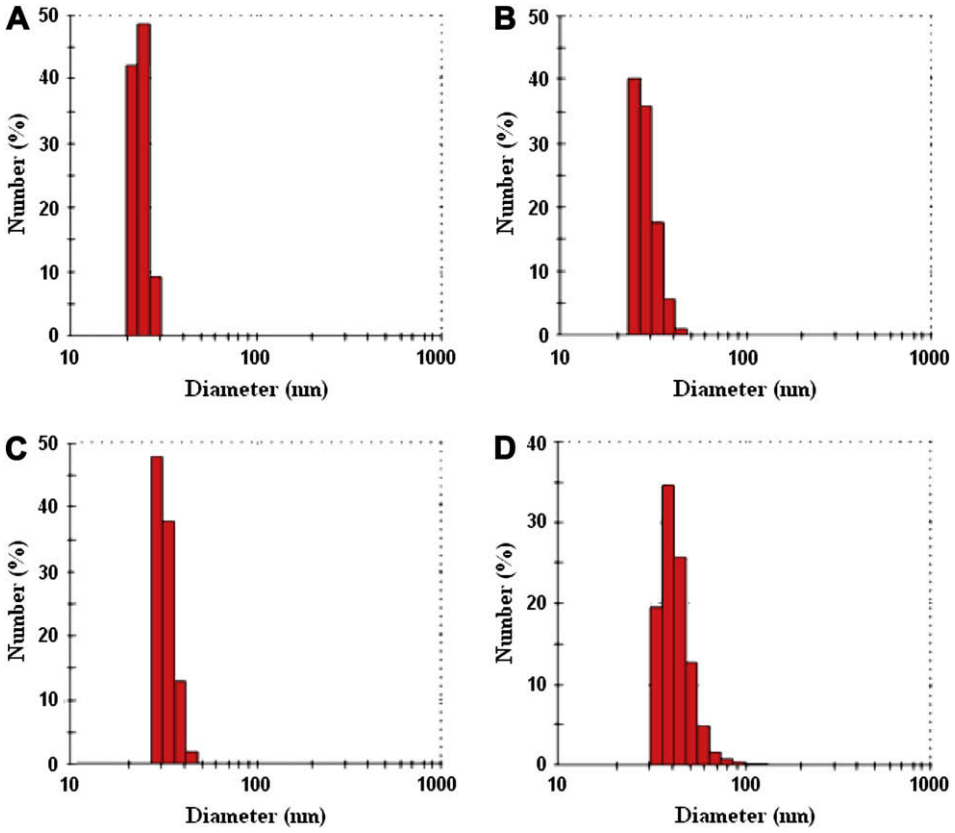

Fig. 6. Size distribution of the self-assembled aggregates determined by DLS: (A) $PAGE_{2k}$/cys-*b*-PLA_{2k}, (B) $PAGE_{2k}$/cys-*b*-PLA_{4k}, (C) $PAGE_{4k}$/cys-*b*-PLA_{4k}, and (D) $PAGE_{2k}$/cys-*b*-PLA_{4k}.

determined and compared with that of PEO_{2k}-*b*-PLA_{4k}. Fig. 7 showed Bel-7402 cells proliferation in the presence of different contents of $PAGE_{2k}$/cys-*b*-PLA_{4k} and PEO_{2k}-*b*-PLA_{4k} assessed by MTT assay. After 48 h of co-culture, $PAGE_{2k}$/cys-*b*-PLA_{4k} showed better compatibility with Bel-7402 cells compared with the control group (without any additive), especially in the case for which the content of $PAGE_{2k}$/cys-*b*-PLA_{4k} was over 0.1 mg/mL ($p < 0.05$, $n = 6$). There was significant dose-dependent for the effect of $PAGE_{2k}$/cys-*b*-PLA_{4k} on Bel-7402 cells, and the maximal value reached 150% compared with that of the control group. In addition, PEO_{2k}-*b*-PLA_{4k} did not show any cytotoxicity to Bel-7402 cells.

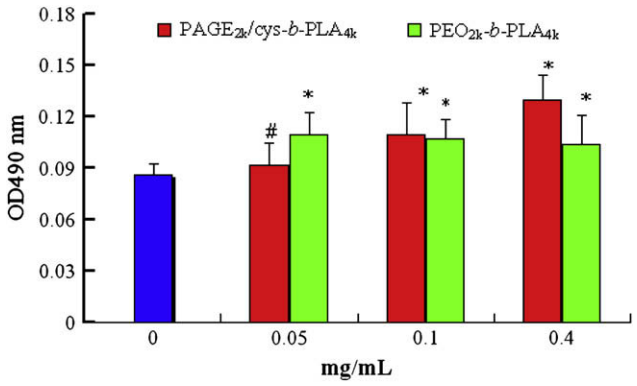

Fig. 7. Effects of $PAGE_{2k}$/cys-*b*-PLA_{4k} and PEO_{2k}-*b*-PLA_{4k} concentrations on the proliferation of human hepatocellular carcinoma Bel-7402 cells, determined by MTT method based on the succinic dehydrogenase activity. The group without any treatment (containing 0 mg/mL of polymers) was chosen as the control. Cells were seed at 10^4 cells/cm^2 and allowed to proliferate for 48 h. The data were expressed as means ± SD ($n = 6$). "*" and "#" mean the significant differences compared with plate control and PEO-b-PLA, respectively ($p < 0.05$).

However, the effect of PEO_{2k}-*b*-PLA_{4k} on the proliferation of Bel-7402 cells represented a constant effect when the contents of PEO_{2k}-*b*-PLA_{4k} varied from 0.05 to 0.4 mg/mL. Therefore, polymeric micelles based on the biodegradable copolymer PAGE/cys-*b*-PLA may be suitable for the development of nanoscale drug delivery systems.

4. Conclusions

In this study, we described a relatively easy and efficient strategy to prepare PLA-based functional amphiphilic block copolymers. The copolymers PAGE-*b*-PLA were synthesized first by ring-opening polymerization of LA with PAGE as macroinitiator and $Sn(Oct)_2$ as catalyst. The amphiphilic block copolymers bearing amino acid residues PAGE/cys-*b*-PLA were obtained by the free-radical addition of cysteine hydrochloride onto the double bonds of PAGE-*b*-PLA. This reaction proceeded completely under mild conditions without degradation of the polymer backbone. These amphiphilic block copolymers can self-assemble into aggregates in aqueous solution. By tuning the hydrophilic and hydrophobic block length within the copolymers PAGE/cys-*b*-PLA, the morphologies of the self-assembly aggregates changed from micelles to vesicles. Moreover, the in vitro cytotoxicity investigation showed that the copolymers PAGE/cys-*b*-PLA exhibited good compatibility with Bel-7402 cells. Therefore, these amphiphilic block copolymers bearing amino acid residues are expected to facilitate a variety of potential biomedical applications.

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