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Synthesis and characterization of functional poly(γ-benzyl-L-glutamate) (PBLG) as a hydrophobic precursor

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ABSTRACT

Four kinds of functional poly(γ-benzyl-L-glutamate) (PBLG) copolymers containing chloro, azido, allyl or propargyl groups on the side chains were synthesized through ester exchange reactions of PBLG with functional alcohols without any protection and de-protection process. Hydrolysis of PBLG, which was found during the ester exchange reaction under low ratios of alcohol to the repeat units of PBLG, was thoroughly investigated, and could be successfully depressed by addition of certain amount of benzyl alcohol to the reaction system. Click chemistry reactions of the azidized or propargylated copolymers, thiol–ene reaction of the allyllated copolymer were taken successfully, indicating that the functional groups on the copolymers were still reactive. Microspheres were also formed, which showed the potential application of the functional polymers as micro-carriers. Characterizations including ^{1}H NMR, ^{13}C NMR, FTIR, elemental analysis, DSC, TGA, ESEM, fluorescence microscope and SEC-MALS were taken.

1. Introduction

Recent years, functional polymers or copolymers have attracted more and more attentions for their important usage in block, graft, branched or ring copolymer synthesis, and biological or medical conjugate [1–7]. Functional polymers or copolymers could be synthesized through polymerization or copolymerization of monomers which had functional groups, such as poly(carbonate-ester)s [8], functional poly(ε-caprolactone) [9], poly(L-serine ester) [10], poly(α-malic acid) [11] and polyester-amides such as poly-(lactic acid-*co*-lysine) [12]. However, the synthesis of some functional monomers was difficult and with low yield, like serine-β-lactone [10] and 3-[N^{ε}-(carbonylbenzoxy)-L-lysyl]-6-L-methyl-2,5-morpholinedione [12], which restricted their further application. The second way is to use the functional molecules as initiators to get polymers with functional end groups. For example, Boc-amino-ethanol was used to initiate L-lactide, after de-protection of Boc, PLLA with amino end group could be obtained [13]. The third way is the functional modification of polymers or copolymers. In this way, the starting polymers mostly were natural polymers, such as chitosan [14], or polymers that could be easily synthesized like PEG [15], PLA [16]. Although the inherent properties of the natural polymers show some limitation, the functional modification of the polymers could be realized when well designed.

Poly(α-amino acid) was a kind of biocompatible and biodegradable synthetic polymer. PBLG is one example of poly(α-amino acid) which is often used as drug delivery carrier and gene vector [17,18]. However, because of the hydrophobic character, uncontrolled degradation and lack of functional groups, the application of PBLG is still limited. Introduction of a second component such as PEG [15], PEI [18], polyester [19], other poly(α-amino acid) [20], or poly [2-(dimethylamino)ethyl methacrylate] (PDMAEMA) [21] to get block, graft or hyper-branched copolymers was an important way to improve PBLG's properties. The other way was the modification of the ester side chains on PBLG, such as acid hydrolysis to get poly-(γ-benzyl-L-glutamate-*co*-L-glutamic acid) [22], aminolysis to get poly [*N*-(2-hydroxyethyl-L-glutamine)] (PHEG) [23] or poly(*N*-hydroxyethyl-L-glutamine-*co*-*N*-hydroxypentyl-L-glutamine) [24]. Ester exchange of PBLG was another convenient way to modify the side chains of PBLG. For example, ester exchange is used to obtain poly(glutamate) with long *n*-alkyl side chains [25], copoly(γ-methyl, benzyl-L-glutamate) [26], and poly(glutamate)-*g*-PEG [27]. During the synthesis, functional groups such as amino, carboxyl, hydroxyl that frequently used must undergo protecting and de-protecting processes, and often bring uncertain reactions, which greatly restrict their application. Recent years, more and more attention was paid to polymers' azidation, alkynylation or allylation [1,4,7,28,29], because the azide/alkyne 1,3-dipolar cycloaddition (click reaction) could be taken in aqueous phase under very mild condition with high efficiency, and thiol–ene reaction could be used in photopolymerization. Although there were reports about functional poly(glutamate) such

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as poly(γ-chloroethyl-L-glutamate) [30] and poly(γ-alkenyl-L-glutamate) [31] synthesized by polymerization of functional glutamate *N*-carboxyl-α-amino acid anhydrides (NCAs), functionalization of poly(glutamate) by ester exchange with functional alcohols such as 2-chloroethanol, 2-azidoethanol would be a more convenient way to get copolymers that has controlled amount of the functional groups on the side chains without protection and de-protection processes, and the hydrophobic character of PBLG is still reserved. In this paper we firstly report the novel synthesis of the functional PBLG by ester exchange reactions using functional alcohols without any protection and de-protection processes. Four kinds of functional PBLGs: partially chlorinated PBLG (PBCLG), partially azidized PBLG (PBN_3LG), partially allyllated PBLG (PBALG) and partially propargylated PBLG (PBPLG) were synthesized, and their structures and properties were thoroughly investigated. The reactivity of the functional groups on PBLG was examined through click chemistry or thiol–ene reaction. This method provided an easy tool to obtain the functional hydrophobic carrier with the capacity to bind with the bioactive materials under mild conditions.

2. Experiment part

2.1. Materials

3-Chloropropanol, propargyl alcohol, propargyl bromide and thioglycol were purchased from Tianzunzezhong chemical limited corporation (Nanjing, China). L-Ascorbic acid sodium salt was obtained from Acros Organics. Rhodamine B was obtained from Sigma. 2,2′-Azobis (2-methylpropionitrile) (AIBN) was recrystallized in methanol and stored at 4 °C. 2-Azidoethanol and 3-azidopropanol were prepared from 2-chloroethanol and 3-chloropropanol according to literatures [32]. Tetrahydrofuran (THF) was dried by refluxing with sodium and then distilled. Ethyl acetate, chloroform and petroleum ether (boiling range: 60–90 °C) were all dried by refluxing with CaH_2 and then distilled. Dried *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were obtained by distillation under reduced pressure after immersed with CaH_2 for more than two weeks. All other reagents were commercially available and used without further purification.

2.2. Instrumentation

^{1}H NMR and ^{13}C NMR spectrums were recorded on a Bruker AVANCE DRX 400 and 300 spectrometers in $CF_3COOD/CDCl_3$ (1/5, v/v) or DMSO-d_6. Fourier transform infrared (FT-IR) spectra were measured with a Bruker Vertex 70 spectrometer on KBr pellets or coating films of chloroform solution. Elemental analysis was performed with a Vario EL elemental analyzer. Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) were measured by Perkin–Elmer at a heating rate of 10 °C/min under nitrogen atmosphere. Size exclusion chromatography – Multi-angle light scattering (SEC-MALS) analysis was performed by combining a Water-515 GPC equipped with Waters Styragel HMW6E column (eluent: 0.1 M LiBr/DMF, flow rate: 1.0 ml/min, at 40 °C), and a DAWN EOS MALS detector (Wyatt Technology, laser wavelength: 690.0 nm). The morphology of microspheres was observed by a field emission environmental scanning electron microscope (ESEM) (Model XL 30 ESEM FEG from Micro FEI Philips). Fluorescence microscope pictures were taken at an excitation wavelength of 555 nm, and took a white light graph as comparison.

2.3. Synthesis of poly(γ-benzyl-L-glutamate) (PBLG)

PBLG was synthesized by polymerization of *N*-carboxyl-α-amino acid anhydride (NCA) of γ-benzyl-L-glutamate in anhydrous chloroform using *n*-hexylamine as an initiator according to literatures [33,34]. The molecular weight of PBLG was determined by ^{1}H NMR.

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 0.94 (t, 3H), 1.35 (br, 8H), 1.59 (br, 2H), 2.06, 2.24 (br d, $J = 72$ Hz, 2H), 2.56 (br, 2H), 4.76 (br, 1H), 5.18 (br, 2H), 7.35 (s, 5H). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 25.2 ($\underline{CH_2}$–COO), 30.1 (CH–(CO)–$\underline{CH_2}$), 55.8 ($\underline{CH}$–CO), 65.5 ($\underline{CH_2}$–Ph), 127.2–128.7 and 136.0 (Ph), 171.6 ($\underline{COOCH_2}$Ph), 175.0 ($\underline{COCH}$). IR (KBr, thin film, cm^{-1}): 3294 (CO–$\underline{NH}$), 1733 ($\underline{COOR}$), 1652 ($\underline{CONH}$), 750, 698 (Ph).

2.4. The ester exchange reactions of PBLG

Functional PBLGs were synthesized by the ester exchange reactions between PBLG and functional alcohols in 1,2-dichloroethane using *p*-toluenesulfonic acid (*p*-TSA) as catalyst according to literatures [25–27]. A typical reaction process was: 1.0 g PBLG (4.56 mmol of benzyl glutamate repeat units) and 0.5 g *p*-TSA (2.63 mmol) were mixed in a reaction bottle with addition of 25 ml 1,2-dichloroethane and a certain amount of 2-chloroethanol. The reaction mixture was kept at 55 °C for 24 h under nitrogen atmosphere. The product was precipitated out in diethyl ether, filtered, and dissolved in a little amount of chloroform, then re-precipitated, filtered, and vacuum-dried to give PBCLG. The degree of Cl substitution was determined by ^{1}H NMR.

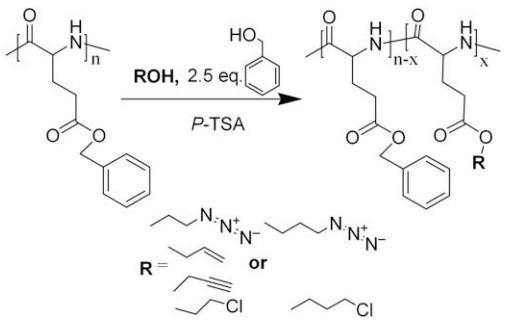
The effect of reaction conditions on ester exchange and hydrolysis was investigated by varying catalyst ratios (from 0 to 1.0, g/g, cat./PBLG), reaction times (from 0 to 24 h), reaction temperatures (from room temperature to 60 °C), ratios of alcohol (using 2-cholоethanol as the model) to PBLG [from 0 equiv to 5.0 equiv (to the amount of the repeat units on PBLG)]. Benzyl alcohol was also added to depress the hydrolysis of PBLG.

2.5. Optimized ester exchange reaction processes

The optimized ester exchange reaction condition and reaction processes were the same as the typical procedure mentioned above [55 °C for 24 h at a catalyst ratio of 0.5/1 (g/g, cat./PBLG)], 2.5 equiv benzyl alcohol was added together with the certain amount of functional alcohols to inhibit the hydrolysis of benzyl ester (see Scheme 1). The typical FTIR and NMR data of all kinds of functional PBLGs were listed below.

2.5.1. Chlorinated PBLG (PBCLG, using 2-chloroethanol)

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 1.98–2.15 (br, d, $J = 72$ Hz, 2H), 2.48–2.55 (br, 2H), 3.50 (br, 2H), 4.28 (br, 2H), 4.63 (br, 1H), 5.13 (br, 2H), 7.30 (s, 5H). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 40.3 ($\underline{CH_2}$–CH_2–Cl), 64.1 ($\underline{CH_2}$–Cl), other signals were the same as PBLG's. IR (KBr, thin film, cm^{-1}): 3301 (CO–$\underline{NH}$), 1739 ($\underline{COOR}$), 1651 ($\underline{CONH}$), 752, 698 (Ph).



Scheme 1. Synthesis of functional PBLGs through ester exchange reactions.

2.5.2. PBC'LG (using 3-chloropropanol instead of 2-chloroethanol)

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 1.96 (br, 2H), 2.06–2.12 (br, d, $J = 72$ Hz, 2H), 2.43–2.47 (br, 2H), 3.54 (br, 2H), 4.27 (br, 2H), 4.69 (br, 1H), 5.11 (br, 2H), 7.31 (s, 5H). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 33.1 ($\underline{CH_2}$–CH_2–Cl), 41.5 ($\underline{CH_2}$–Cl), 61.0 ($\underline{CH_2}$–CH_2–CH_2–Cl), other signals were the same as PBLG's. IR (KBr, thin film, cm^{-1}): 3290 (CO–$\underline{NH}$), 1734 ($\underline{CO}$OR), 1654 ($\underline{CO}$NH), 752, 699 (Ph).

2.5.3. Azidized PBLG (PBN$_3$LG, using 2-azidoethanol)

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 1.98–2.15 (br, d, $J = 72$ Hz, 2H), 2.48–2.55 (br, 2H), 3.50 (br, 2H), 4.28 (br, 2H), 4.63 (br, 1H), 5.11 (br, 2H), 7.30 (s, 5H). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 49.2 ($\underline{CH_2}$–N_3), 62.7 ($\underline{CH_2}$–CH_2–N_3), other signals were the same as PBLG's. IR (KBr, thin film, cm^{-1}): 3293 (CO–$\underline{NH}$), 2108 ($\underline{N_3}$–), 1737($\underline{CO}$OR), 1655, 1626 ($\underline{CO}$–NH), 751, 698 (Ph).

2.5.4. PBN$_3$′LG (using 3-azidopropanol)

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 1.98–2.18 (br, d, $J = 72$ Hz, 4H), 2.5–2.6 (br, 2H), 3.4 (br, 2H), 4.24 (br, 2H), 4.69 (br, 1H), 5.11 (br, 2H), 7.3 (s, 5H). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 27.5 ($\underline{CH_2}$–CH_2–N_3), 47.5 ($\underline{CH_2}$–N_3), 61.1 ($\underline{CH_2}$–CH_2–CH_2–N_3), other signals were the same as PBLG's. IR (KBr, thin film, cm^{-1}): 3293 (CO–$\underline{NH}$), 2099 ($\underline{N_3}$–), 1736 ($\underline{CO}$OR), 1655 ($\underline{CO}$NH), 751, 698 (Ph).

2.5.5. Allylated PBLG (PBALG, using allyl alcohol)

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 1.99–2.17 (br, d, $J = 72$ Hz, 2H), 2.47–2.53 (br, 2H), 4.61 (br, 2H), 4.70 (br, 1H), 5.12 (br, 2H), 5.25 (br, 2 H), 5.83 (br, 1H), 7.32 (s, 5H). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 64.4 ($\underline{CH_2}$–CH=$\underline{CH_2}$), 117.4 (CH=$\underline{CH_2}$), 132.5 ($\underline{CH}$=CH_2), other signals were the same as PBLG's. IR (KBr, thin film, cm^{-1}): 3295 (CO–$\underline{NH}$), 1736 ($\underline{CO}$OR), 1655 ($\underline{CO}$NH), 991, 936 (CH=$\underline{CH_2}$), 752, 699 (Ph).

2.5.6. Propargylated PBLG (PBPLG, using propargyl alcohol)

^{1}H NMR (400 MHz, $CF_3COOD/CDCl_3$, δ, ppm): 2.00–2.18 (br d, $J = 72$ Hz, 2H), 2.47–2.55 (br, 3H), 4.66–4.69 (br, 3H), 5.13 (br, 2H), 7.32 (s, 5H). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 51.7 ($\underline{CH_2}$–C≡CH), 77.2 (–C≡$\underline{CH}$), 78.2 (–$\underline{C}$≡CH), other signals were the same as PBLG's. IR (KBr, thin film, cm^{-1}): 3294 ($\underline{CONH}$ and –C≡$\underline{CH}$), 2128 (–$\underline{C}$≡$\underline{CH}$), 1738 ($\underline{CO}$OR), 1654 ($\underline{CO}$NH), 738, 698 (Ph).

2.6. Click reactions

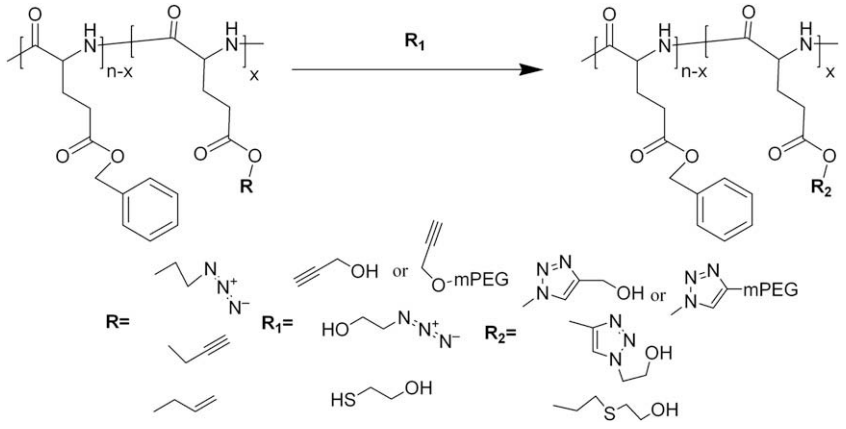
PBN$_3$LG reacted with propargyl alcohol or propargyl mPEG2000 (prepared according to literature [35]) through click reactions using $CuSO_4 \cdot 5H_2O$ (0.1 equiv to N_3 group) and L-ascorbic acid sodium salt (0.11 equiv to N_3 group) as catalyst in DMSO at 80 °C for 6 h or overnight, respectively (Scheme 2). The reaction mixture was poured into water and filtered. The precipitant was washed with methanol and ether, then dried under vacuum to get PBN$_3$PLG (for propargyl mPEG2000, the reaction mixture was dialyzed against DMSO using a dialysis tube with M_w cut-off of 7000 Da for 3 days, most of DMSO was removed under reduced pressure at about 85 °C, then the product was precipitated out in diethyl ether, filtered, and vacuum-dried to give PBN$_3$LG-*g*-mPEG.); The same reaction procedure as that of PBN$_3$LG with propargyl alcohol was carried out for PBPLG with 2-azidoethanol to get PBPN$_3$LG.

2.7. Thiol–ene reaction

PBALG (0.05 g, containing 0.085 mmol of allyl groups) was added to a reaction flask and dried by toluene azeotropic distillation, then thioglycol (0.25 ml, 40 equiv to allyl groups), AIBN (0.028 g, 2.0 equiv to allyl groups) and dried DMF (2 ml) were added. After sealing the flask, the reaction mixture was kept at 80 °C for 24 h under argon atmosphere [36], then poured into water. The precipitant was filtered and washed with diethyl ether, then dried under vacuum to get PBALG-s-OH.

2.8. Preparation of functional PBLG microspheres

PBN$_3$LG was used as an example. PBN$_3$LG microspheres were prepared by O/W emulsion method. First, PBN$_3$LG (0.1 g) was dissolved in 1,2-dichloroethane/DMAc (5 ml, v/v = 4:1), then the solution was dispersed in 1.0 wt% of poly(vinyl alcohol) solution (100 ml) by high-shear dispersing emulsifier at a speed of 2000 rpm for 2–3 min. The O/W emulsion was stirred at 40 °C for 12–24 h to evaporate the solvent. After that, the microspheres were collected by centrifugation and washed several times with water and hot water, and then freeze-dried. Rhodamine B (0.2 g, 0.42 mmol) was reacted with an excess amount of propargyl alcohol (36.5 μl, 1.26 mmol) in dried DMSO (10 ml) using dicyclohexyl carbodiimide (DCC) (0.128 g, 0.42 mmol) and 4-dimethylaminopyridine (DMAP) (0.025 g, 0.21 mmol) as catalyst system. The reaction mixture was precipitated in and washed with diethyl ether to remove excess propargyl alcohol. After vacuum dried, alkynyl-Rhodamine B was gotten. Alkynyl-Rhodamine B was conjugate to PBN$_3$LG microspheres through click chemistry in water at 37 °C for 24 h using $CuSO_4 \cdot 5H_2O$ (0.1 equiv to alkynyl-Rhodamine B) and L-ascorbic acid sodium salt (0.11 equiv) as catalyst. The reaction mixture was dialyzed against water using a dialysis tube with M_w cut-off of 7000 Da for 3 days and then freeze-dried to obtain



Scheme 2. Click reactions and thiol–ene reaction of functional PBLGs.

Rhodamine B loaded PBN_3LG microspheres. The microspheres were characterized by ESEM and fluorescence microscope.

3. Results and discussion

3.1. Synthesis of PBLG and the ester exchange reaction of PBLG with 2-chloroethanol

PBLG could be conveniently synthesized through ring opening polymerization of γ-benzyl-L-glutamate NCA. The polymerization degree of PBLG was about 66 (by ^{1}H NMR), and the molecular weight was about 14.4 kDa.

Generally, ester exchange reactions of PBLG or poly(γ-methyl-L-glutamate) (PMLG) were used to get poly(glutamate) with long *n*-alkyl side chains [25], copoly(*r*-methyl, benzyl-L-glutamate) [26] or poly(glutamate)-*g*-PEG [27]. Some work [30,31] has mentioned about the functional poly(glutamate)s by polymerization of functional glutamate NCAs. However, ester exchange between poly(glutamate) and functional alcohols, such as 2-chloroethanol, would be a more convenient way to get functional poly(glutamate)s with many functional groups on the side chains without any protection and de-protection processes. Interestingly, during the ester exchange reaction at low ratios of 2-choloethanol to PBLG (for example, 1.5 equiv to the repeat units on PBLG), hydrolysis of PBLG was found, which was confirmed by the COOH signal at 12.1 ppm of PBCLG's ^{1}H NMR (Fig. 1a). Up to now, few papers mentioned or paid enough attention to the hydrolysis of poly(glutamate) associated with ester exchange. In fact, most of the reports about ester exchange of poly(glutamate) were using PMLG. The methyl ester was more stable than benzyl ester under acidic environment. In our experiment, when PBLG was mixed with the catalyst (*p*-TSA) alone at 55 °C for 24 h, hydrolysis of PBLG occurred, which was confirmed by the signal of COOH at 12.1 ppm by ^{1}H NMR (Fig. 2b) and elemental analysis (C/N ratio decreased from 10.79 to 9.75). Furthermore, alcohols or mPEG that used in the literatures for ester exchange were large excess (more than 20 equiv to poly(glutamate) [25–27]). We assumed that acid (in our case, *p*-TSA) would be an important factor to induce the hydrolysis of PBLG, and hydrolysis and ester exchange were a pair of competitive reactions. When the alcohol used in the ester exchange with poly(glutamate) was large excess, the hydrolysis could be inhibited. However, at lower ratios of alcohol to PBLG, hydrolysis would occur in some extent. This assumption will be discussed in detail in the subsequent chapters. In fact, if the ratio of 2-chloroethanol to PBLG was larger than 5.0 equiv, hydrolysis of PBLG would not be observed in our experiments (see Fig. 3d). However, too many functional groups by using large excess of functional alcohols would be commonly unnecessary. It's not worthwhile in economy when the starting material is expensive. What's more, because of the difference of steric hindrance, not all the alcohols would have the same reactivity. Thus, the investigation on the hydrolysis of PBLG during ester exchange reaction under low ratios of alcohol to PBLG becomes very important and necessary.

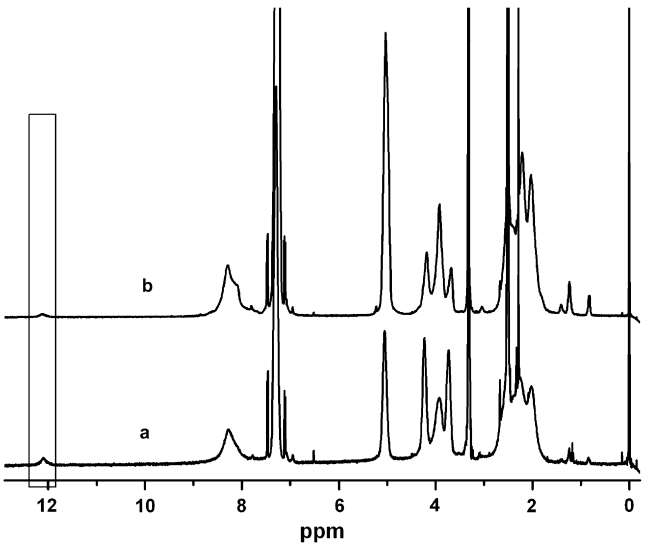


Fig. 1. ^{1}H NMR spectrums of PBCLGs obtained from ester exchanges between PBLG and 2-chloroethanol with or without addition of benzyl alcohol [a. only addition of 2-chloroethanol (1.5 equiv to the amount of the repeat units on PBLG); b. addition of 2-chloroethanol (1.5 equiv) and benzyl alcohol (2.5 equiv)].

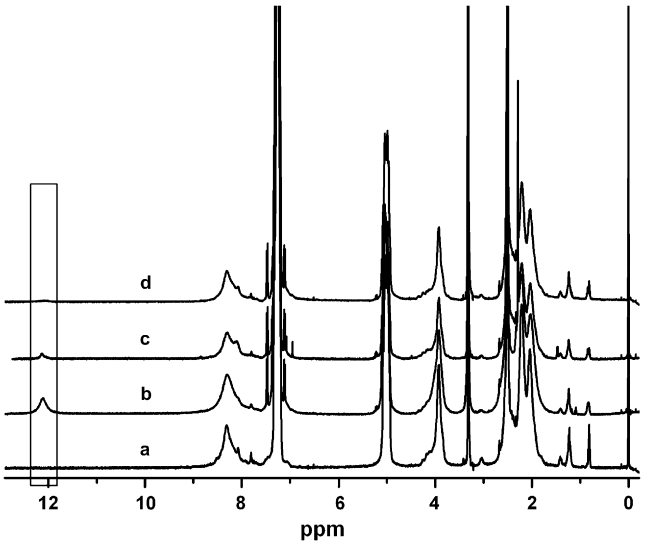


Fig. 2. ^{1}H NMR spectrums of PBLG reacted with *p*-TSA (PBLG/*p*-TSA: 1/0.5, g/g) and different amount of benzyl alcohol for 24 h [a. original PBLG; b. without benzyl alcohol; c. addition of benzyl alcohol (1.0 equiv to the amount of the repeat units on PBLG); d. addition of benzyl alcohol (2.5 equiv)].

3.2. The effect of reaction conditions on ester exchange and hydrolysis

The effect of reaction conditions on ester exchange and hydrolysis was investigated using 2-chloroethanol as the model. In this reaction, 2-chloroethanol was attached to side chain of PBLG via ester exchange reaction with the original benzyl ester group. Here, we use the degree of Cl substitution on PBLG to represent the degree of ester exchange.

It was found that, the degree of Cl substitution and hydrolysis all increased while increasing catalyst ratio, and the Cl substitution kept stable when catalyst ratios were larger than 0.5 g/g (Fig. 3a). It's obvious that the reactivity of ester exchange would increase with the increase amount of catalyst over a certain range. However, since the catalyst is acidic, the reactivity of hydrolysis would increase too. The degree of Cl substitution increased with prolonged reaction time, and after 24 h, the ester exchange reaction was almost stable [26]. At the same time, the degree of hydrolysis kept decreased (Fig. 3b). The degree of Cl substitution increased, while the degree of hydrolysis decreased with the reaction temperature rising from 25 °C to 55 °C. Higher reaction temperature than 55 °C would bring little change to the system (Fig. 3c); Increasing the ratio of 2-chloroethanol to PBLG would promote the conversation of ester exchange, meanwhile, the hydrolysis degree kept decreasing, and when the ratio of 2-chloroethanol to PBLG reached to 5.0, no hydrolysis could be confirmed (Fig. 3d). It's implied that ester exchange and hydrolysis were a pair of competitive reactions existing in the reaction procedure. We assumed that hydrolysis of PBLG could happen under acidic condition

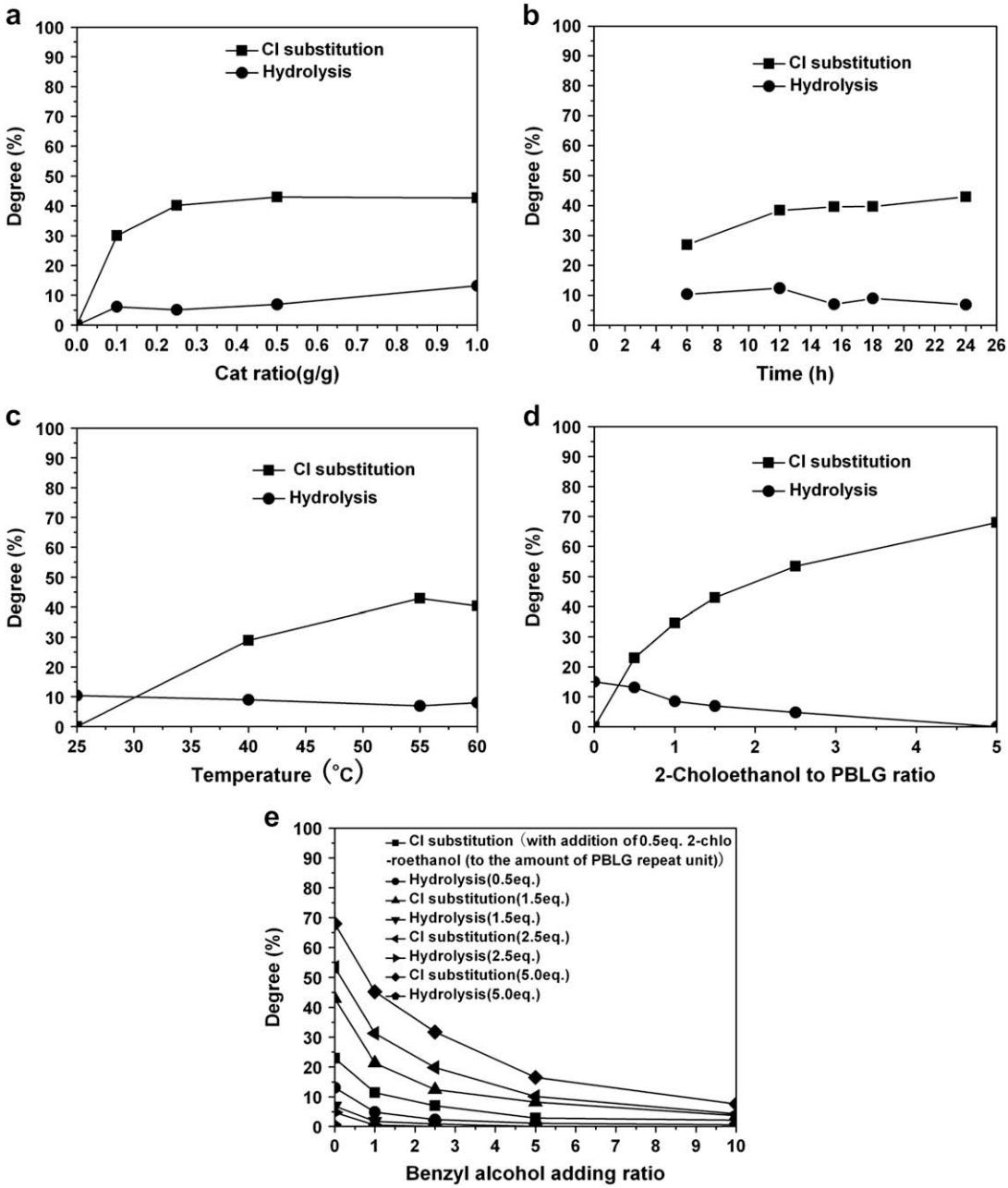


Fig. 3. Effect of reaction conditions on ester exchange and hydrolysis of PBLG [a. changing catalyst ratio (cat./PBLG, g/g); b. changing reaction time; c. changing reaction temperature; d. changing molar ratio of 2-chloroethanol to PBLG (repeat units); e. changing additional molar ratio of benzyl alcohol to PBLG (repeat units)].

at room temperature, and had little dependence on reaction temperature. However, rising temperature could strongly increase the reactivity of ester exchange, leading to the inhibition of the hydrolysis at the same time. Under this condition, prolonging reaction time was beneficial to ester exchange reaction, resulted in the higher conversation of the ester exchange. It's noticed that hydrolysis of PBLG is an equilibrium reaction, determined by the concentrations of acid (like *p*-TSA) and alcohol in the system. To increase the ratio of alcohol to PBLG, the equilibrium would tend to shift to the reverse direction, by which the hydrolysis reaction would be depressed. We chose benzyl alcohol as an additional component to the ester exchange reaction mixture. It's not only to increase the alcohol concentration to inhibit hydrolysis, but also to keep the structure of PBLG copolymer unchanged. In fact, no hydrolysis of PBLG could be confirmed by the ^{1}H NMR, when 2.5 equiv (to the repeat units on PBLG) benzyl alcohol was added to the system (without 2-chloroethanol in the system) (Figs. 2d and 3e). It's worthwhile to mention that benzyl alcohol also took part in the ester exchange reaction, which brought loss of the ester exchange degree (Fig. 3e).

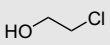
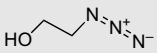
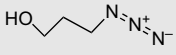
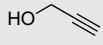
3.3. Functional PBLGs and their properties

According to the above investigation and discussion, the optimized ester exchange reaction condition was concluded. PBLG was reacted with functional alcohols and 2.5 equiv of benzyl alcohol together under 55 °C for 24 h at a catalyst ratio of 0.5/1 (cat./PBLG, g/g), and four kinds of partially functionalized PBLGs (see Scheme 1) were obtained by using relatively low excess of functional alcohols (see Table 1). The structures of the synthesized copolymers were confirmed by ^{1}H NMR, ^{13}C NMR and FTIR (see Experiment part).

All the functional PBLGs have the good solubility in strong polar solvents such as DMSO, dimethylacetamide (DMAc), DMF, THF, and most of functional PBLGs (except for azidized PBLGs (PBN_3LG and $PBN_3'LG$) are also soluble in 1,2-dichloroethane and chloroform. The molecular weights of functional PBLGs were determined by ^1H NMR and SEC-MALS. The result showed that the molecular weight of functional PBLGs changed little in comparison with that of unmodified PBLG (Table 2), because of the inconspicuous differences of the molecular weights between functional alcohols used for ester exchange and the original benzyl groups on the side chains of PBLG. It is also implied that no degradation of the back bone of PBLG happened during the ester exchange reaction. Since mPEG was also a polymer, PBN_3LG-*g*-mPEG had a larger molecular weight than that of PBLG. SEC-MALS analysis clearly indicated the increase of the molecular weight of PBN_3LG-*g*-mPEG in comparison with PBN_3LG, and their polydispersities were very narrow (Fig. 4). From the ^1H NMR of PBN_3LG-*g*-mPEG (Fig. 5), the amount of the grafted mPEG was calculated to be 14.1 mPEG chains per PBLG, with a substitution degree of about 21.4% on PBLG. The graft efficiency was about 94%. All the polydispersities of the functional PBLGs were close to 1.0, indicating that the ester exchange reaction was well under control.

The glass transition temperature (T_g) of the functional PBLGs varied with different functional groups on the side chains as shown in Table 1. PBCLG (Table 1, entries a1–a3) showed a T_g close to that of original PBLG (19.4 °C), indicating that this extend of ester exchange using 2-chloroethanol did not have much change on the flexibility of polymer chain. However, increasing a methylene group to the functional alcohols (using 3-chloropropanol instead of 2-chloroethanol) would clearly decrease the rigidity of the side chains, leading to the obvious decrease of T_g (Table 1, entries a′1–a′3). It should be mentioned that the substitution degrees also varied with the different kind of alcohols under the same reaction condition, even the starting ratios of alcohol/PBLG were the same. Since samples b′1–b′3 had the obviously higher substitution degrees than samples b1–b3 at the same alcohol/PBLG ratios, and also the side chains of b′1–b′3 were longer than that of b1–b3, the further decrease of T_g of $PBN_3'LG$ than that of PBN_3LG is understandable (Table 1, entries b1–b3, b′1–b′3). The existence of the azido groups seems to have the great contribution to the much lower T_gs of PBN_3LG and $PBN_3'LG$ than that of the original PBLG. It was reported that azido esters were often used in plasticization area to obtain polymer materials with good mechanical properties and lower T_gs [37]. An in-depth investigation is probably needed. The lower T_gs of PBALGs (Table 1, entries c1–c3) than that of original PBLG was possibly due to the less steric hindrance of the allyl group than that of benzyl groups. The T_gs of PBPLGs were a little bit higher than that of PBLG (Table 1, entries d1–d3). It was implied that the linear propargyl groups had the obvious rigidity which restricted the free movement of the polymer chain.

Table 1
Ester exchange of PBLG.[a]

Entry	Alcohol	Alcohol/PBLG ratio (equiv to the repeat units on PBLG)	Degree[b] of substitution/ (hydrolysis) (%)	T_g[c]/T_d[c] (°C)
a1		1.5	12.4 (0.9)	19.9/265.7
a2		2.5	19.8 (0)	19.5/267.2
a3		5.0	31.7 (0)	20.9/260.3
a′1		1.5	29.7 (0.3)	0.7/264.2
a′2		2.5	38.6 (0)	6.6/260.9
a′3		5.0	55.8 (0)	2.2/259.2
b1		1.5	14.0 (0.1)	16.0/249.6
b2		2.5	22.8 (0)	12.6/254.6
b3		5.0	35.4 (0)	7.6/244.6
b′1		1.5	27.3 (0)	3.6/254.0
b′2		2.5	37.5 (0)	−2.8/236.2
b′3		5.0	49.5 (0)	−12.4/233.6
c1		1.5	24.4 (0)	11.1/261.9
c2		2.5	34.2 (0)	8.7/266.8
c3		5.0	45.4 (0)	9.6/265.8
d1		1.5	12.1 (0)	21.0/254.0
d2		2.5	19.8 (0)	21.9/258.3
d3		5.0	29.5 (0)	23.5/255.5

[a] For all the ester exchange reactions, catalyst (*p*-TSA) ratio was 0.5 to PBLG (g/g), temperature:55 °C, time:24 h. Benzyl alcohol (2.5 equiv to the repeat units on PBLG) was also added to the reaction mixture.

[b] By ^1H NMR.

[c] T_g was determined by DSC (second heating scan), and thermal decomposition temperature (T_d) was recorded by TGA at a temperature of 5.0% decomposition (T_g and T_d of the original PBLG were 19.4 °C and 308.0 °C, respectively).

Table 2
Molecular weight of PBLG and functional PBLGs.

Samples	Functional group content (mol%)	M_n (10^4) ^1H NMR	M_n (10^4) SEC-MALS	M_w/M_n SEC-MALS
PBLG	0	1.44	2.24	1.02
PBCLG (a2)	19.8	1.41	2.10	1.02
PBC′LG (a′2)	38.6	1.41	1.39	1.03
PBN_3LG (b2)	22.8	1.41	1.98	1.02
$PBN_3'LG$ (b′2)	37.5	1.43	2.23	1.04
PBALG (c2)	34.2	1.33	2.22	1.08
PBPLG (d2)	19.8	1.38	2.44	1.03
PBN_3LG-*g*-mPEG	21.4	4.32	7.13	1.07

The TGA results of functional PBLGs were shown in Table 1. All of the functional PBLGs had a lower thermal decomposition temperature (T_d) than that of PBLG (308 °C), showing that the functional ester side chains were more unstable than benzyl ester, and would be decomposed first from the PBLG back bone.

3.4. Verification of the reactivity of functional groups on functional PBLGs

After ester exchange of PBLG, whether these functional side groups still remained the activity for the further application was still a question. Click reactions and thiol–ene reaction which had the high selectivity were carried out to demonstrate the reactivity of azide, propagyl and allyl groups on functional PBLGs.

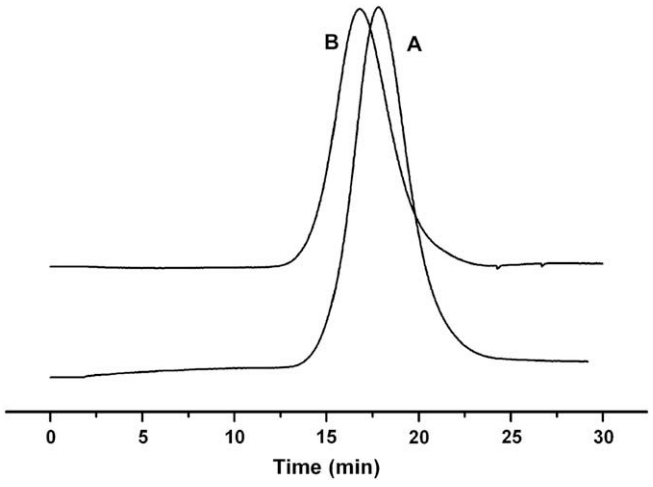


Fig. 4. Molecular weight change of PBN_3LG by click reaction with propargylated mPEG using SEC-MALS analysis (A. PBN_3LG, before reaction; B. PBN_3LG-*g*-mPEG, after reaction).

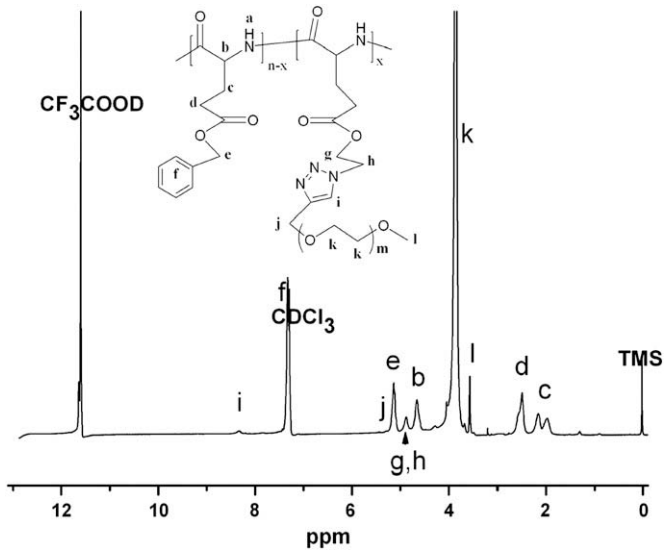


Fig. 5. ^{1}H NMR spectrum of PBN_3LG-*g*-mPEG.

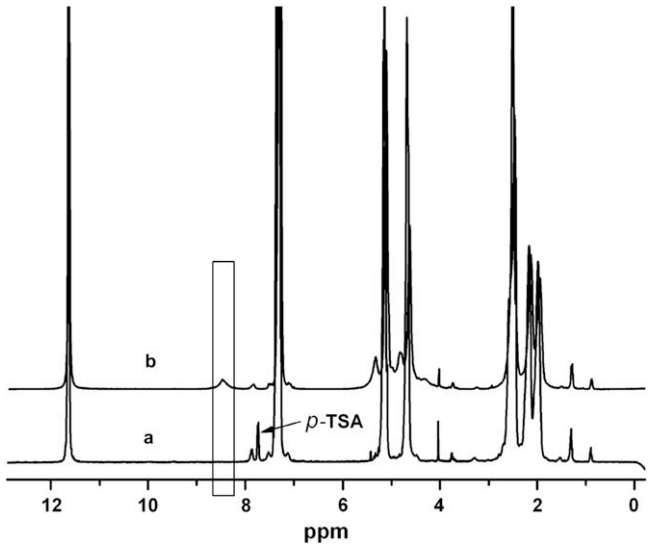


Fig. 7. Change of the ^{1}H NMR spectrums of PBPLG through click reaction with 2-azidoethanol [a. before reaction; b. after reaction ($PBPN_3LG$)].

The peaks on ^{1}H NMR spectrums of PBN_3PLG and $PBPN_3LG$ at 8.3 ppm assigned to the hydrogen of the 1,2,3-triazole ring were the important evidence of the occurrence of click reactions between PBN_3LG with propargyl alcohol and PBPLG with 2-azidoethanol, respectively (Figs. 6 and 7). Meanwhile, the absorbance of N_3 groups at 2108 cm^{-1} on FT-IR spectrum almost disappeared (data not given), indicating that the click reactions were taken successfully with high efficiency. We have mentioned before that propargyl mPEG2000 could graft to PBN_3LG via click reaction with a substitution degree of 21.4%, proved by the significant increase of the M_w from SEC-MALS (Fig. 4) and the peak at 3.8 ppm (methylene group of mPEG) on the ^{1}H NMR of PBN_3LG-*g*-mPEG (Fig. 5).

Comparing with the click reactions, the thiol–ene reaction between PBALG and thioglycol was not so efficient. Although thioglycol and AIBN were large excess, there were still some allyl groups remained from the ^{1}H NMR of PBALG-s-OH (Fig. 8). The graft ratio of thioglycol on PBLG was calculated as about 9.8%, and the remained ratio of allyl groups on PBLG was about 9.9%. The steric hindrance of benzyl groups might be one of the reasons for the low reactivity of the allyl groups.

Anyway, the results clearly indicate that the functional groups on PBLG copolymers coming from the ester exchange reactions maintained enough activities, and could be used for further modifications as the active ends on the polymers.

3.5. Characterization of microspheres

As a kind of hydrophobic polymer, PBLG has been used to make porous spheres by emulsion method [38] as the packing materials for gel permeation chromatography (GPC). The spheres could be further modified with diaminoalkanes and used for endotoxin removal [39]. Since PBLG was partially modified by the functional alcohols, the remained hydrophobic character made it still possible to form microspheres. As a model, PBN_3LG was used to make the microspheres through o/w emulsion method, and the microspheres

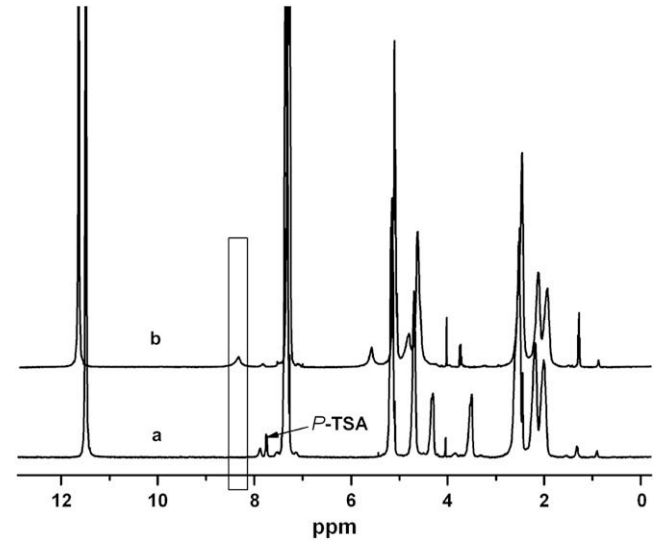


Fig. 6. Change of the ^{1}H NMR spectrums of PBN_3LG through click reaction with propargyl alcohol [a. before reaction; b. after reaction (PBN_3PLG)].

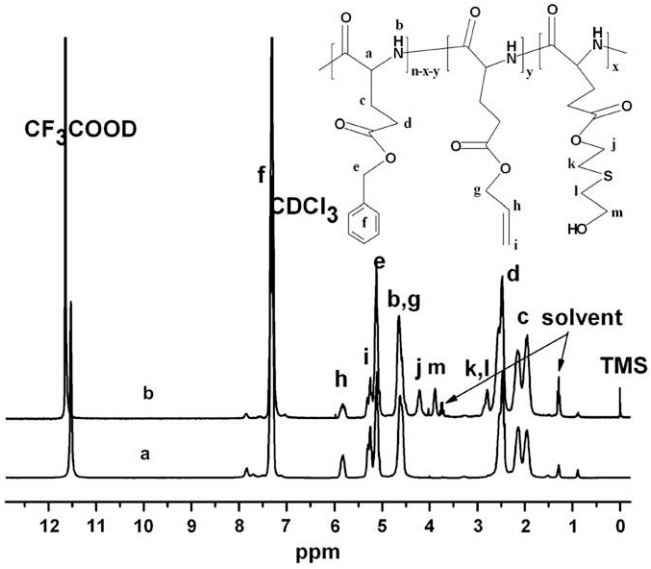


Fig. 8. Change of the ^{1}H NMR spectrums of PBALG through thiol–ene reaction with thioglycol [a. before reaction; b. after reaction (PBALG-s-OH)].

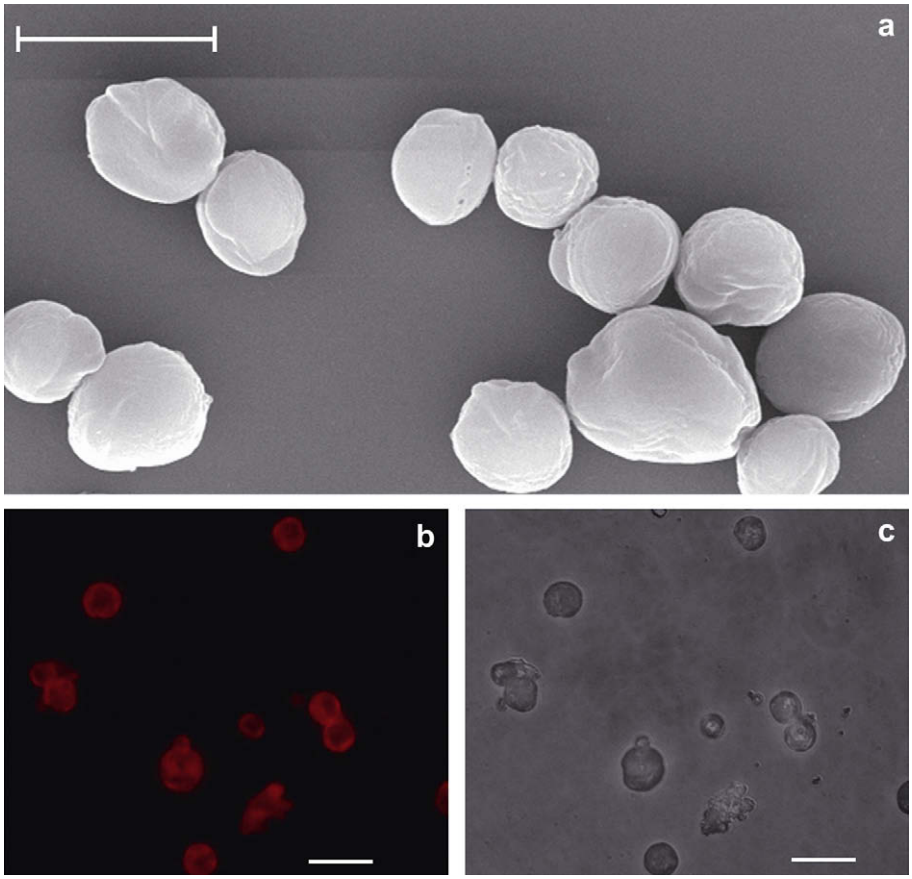


Fig. 9. Morphology observation of the PBN_3LG microspheres (bar = 10 μm; a. ESEM image; b. fluorescence microscope image of Rhodamine B loaded PBN_3LG microspheres; c. the same area as picture B under white light).

were characterized by ESEM (Fig. 9a). The morphology of the microspheres was uniform as the round particles with an average diameter of about 5 μm. Rhodamine B is a widely used fluorescent dye. In order to investigate the distribution situation of the functional groups, Rhodamine B was immobilized onto the PBN_3LG microspheres as fluorescent labeling. Before that, Rhodamine B was first modified with propargyl alcohol via DCC condensation method to obtain an alkynyl group, and then bound to the PBN_3LG microsphere through click reaction. The fluorescence microscopic picture (Fig. 9b and c) clearly showed the red fluorescence (coming from Rhodamine B) covering the surface of the PBN_3LG microspheres, which implied that there were a lot of azide groups spread out over the surface of the PBN_3LG microspheres keeping their reactivities as well. This kind of PBLG copolymer microspheres with many functional and active groups on the surface would have the great potential to be used in the biomedical area, such as drug-carrier, adsorption material, separation and purification medium, long-acting tracer, and diagnostic substrate, etc.

4. Conclusions

A series of partially functionalized PBLGs were successfully synthesized through ester exchange reactions of PBLG with functional alcohols. Hydrolysis of PBLG was successfully depressed by adding a certain amount of benzyl alcohol to the reaction system. The functional groups of the copolymers were active and could be further modified. The functional PBLGs could also form microspheres with functional groups on the surface as the novel bioactive micro-materials.

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