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Factors of Influencing the Grafting Ratio of Poly(γ-benzyl-*L*-glutamate)-*graft*-Poly (ethylene glycol) Copolymer

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Poly(γ-benzyl-*L*-glutamate)-*graft*-poly(ethylene glycol) (PBLG-*graft*-PEG) copolymer was synthesized by the ester exchange reaction of PBLG with PEG. Nuclear magnetic resonance (NMR) spectroscopy and scanning electron microscopy (SEM) were used to characterize the structure of PBLG-*graft*-PEG copolymer. The effects of reaction temperature, reaction time, and the chain length of PEG on the grafting ratio of PBLG-*graft*-PEG copolymer were investigated.

Keywords: poly(γ-benzyl-*L*-glutamate)-*graft*-poly(ethylene glycol), factor; effect, grafting ratio

1 Introduction

In recent years, polypeptide has received much attention both experimentally and theoretically (1–27). Due to the unique structures and properties, synthesized polypeptides and their copolymers have been widely studied in the fields of protein simulation, macromolecular conformational research, catalysis, site-specific drug delivery systems, nanoreactors, etc. (1–6).

Because of the amphiphilc characteristics, block and graft polypeptide copolymers composed of hydrophobic segments and hydrophilic chains could form micelles or nanoparticles with a core-shell structure (1, 7–13). Kwon et al. have reported that poly(β-benzyl *L*-aspartate) (PBLA)/poly(ethylene oxide) (PEO) diblock copolymers could self-assemble to form polymeric micelles with an outer shell of PEO and an inner core of PBLA in aqueous medium (12). Cho et al. reported the formation of polymeric micelles composed of poly(γ-benzyl *L*-glutamate) and poly(ethylene glycol) in aqueous medium and the drug delivery system based on the core-shell nanoparticles with PBLG as the hydrophobic inner core and PEG as the hydrophilic outer shell (9). Harada et al. have studied the relationship between the conformation of the polypeptide segment and the supramolecular structure of poly(*L*-lysine)-*block*-poly(ethylene oxide). It was revealed that the α-helix structure of polypeptide chains tend to be stabilized by the PEO segments through the formation of a dimer with a micelle-like structure in aqueous medium (7).

However, to our knowledge, little experimental work on the study of the factors of influencing the grafting ratio of poly(γ-benzyl-L-glutamate)-*graft*-poly(ethylene glycol) copolymer has been reported so far. In the present study, poly(γ-benzyl-*L*-glutamate)-*graft*-poly(ethylene glycol) (PBLG-*graft*-PEG) copolymer has been synthesized. NMR was used to determine the components of PBLG-*graft*-PEG copolymer. SEM was used to characterize the microstructure of PBLG-*graft*-PEG, and the microstructure was used to compare with that of PBLG homopolymer. The effects of reaction temperature, reaction time, and the chain length of PEG on the grafting ratio of copolymer were studied.

2 Experimental

2.1 Materials

Poly(ethylene glycol methyl ether)s (mPEG) were purchased from Sigma Inc., and used without further purification. Triethylamine, hexane, tetrahydrofuran (THF) and 1, 4-dioxane are of analytical grade and dried with sodium

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to remove water before use. All other solvents are of analytical grade and used without further purification.

2.2 Syntheses of PBLG Homopolymer and PBLG-graft-PEG Copolymer

The PBLG sample was synthesized by a standard *N*-carboxyl-γ-benzyl-*L*-glutamate anhydride (NCA) method (1, 17–19). PBLG was obtained by the ring-opening polymerization of γ-BLG NCA initiated by triethylamine in 1,4-dioxane at room temperature for 72 h. The reaction mixture was poured into a large volume of anhydrous ethanol. The precipitated product was dried under vacuum and then purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol. The molecular weight of PBLG was estimated from the intrinsic viscosity measured in dichloroacetic acid (DCA) (24). The molecular weight of PBLG synthesized was 51000.

PBLG-*graft*-PEG copolymers were obtained by the ester exchange reaction of PBLG hompolymer with mPEG in 1, 2-dichloroethane with *p*-toluenesulfonic acid as a catalyst according to the method described in the documents (1, 19, 26, 27). The mixture reacted at 52–58°C for 1–4 days, it was then precipitated into a large volume of anhydrous ethanol. The resulting product was purified twice by repeated precipitation from a chloroform solution in a large volume of anhydrous methanol, and then dried under vacuum. The molecular weight of PBLG used in the reaction was 51000. The molecular weights of mPEG used in the study were 350, 750, and 1300, respectively, and the corresponding PBLG-*graft*-PEG copolymers synthesized were denoted as PBLG-*g*-PEG1, PBLG-*g*-PEG2, PBLG-*g*-PEG3, respectively. The PBLG/mPEG mol ratio is kept at 1:30.

2.3 ^{1}H-NMR Measurements

^{1}H-NMR spectrum of PBLG-*graft*-PEG was measured in $CDCl_3$ using an NMR instrument (Avance 550) at 500 MHz. The percentage of grafting was calculated from the peak intensities of the methylene proton signal of PBLG and the ethylene proton signal of PEG in the ^{1}H-NMR spectrum.

2.4 SEM Photomicrographs

Testing specimens were prepared by casting films from 30% polypeptide solution in chloroform onto clean glass plates and drying them under vacuum at 50°C. Gold was sprayed on samples in vacuum. Investigation was carried out using a scanning electron microscope (Sirin 200, FEI, Holland). Acceleration voltage was 10 kV and photographs of cross-section of PBLG and PBLG-*graft*-PEG were taken.

3 Results and Discussion

3.1 ^{1}H-NMR Analysis

Figure 1 shows the ^{1}H-NMR spectrum of PBLG-*graft*-PEG1 in $CDCl_3$, where the grafting ratio of copolymer is 26.2%. As seen from Figure 1, the characteristic peaks appearing at 7.26 ppm and 5.03 ppm (corresponding to the phenyl protons in the PBLG segments and the methylene protons in the benzyl group of the PBLG segments,

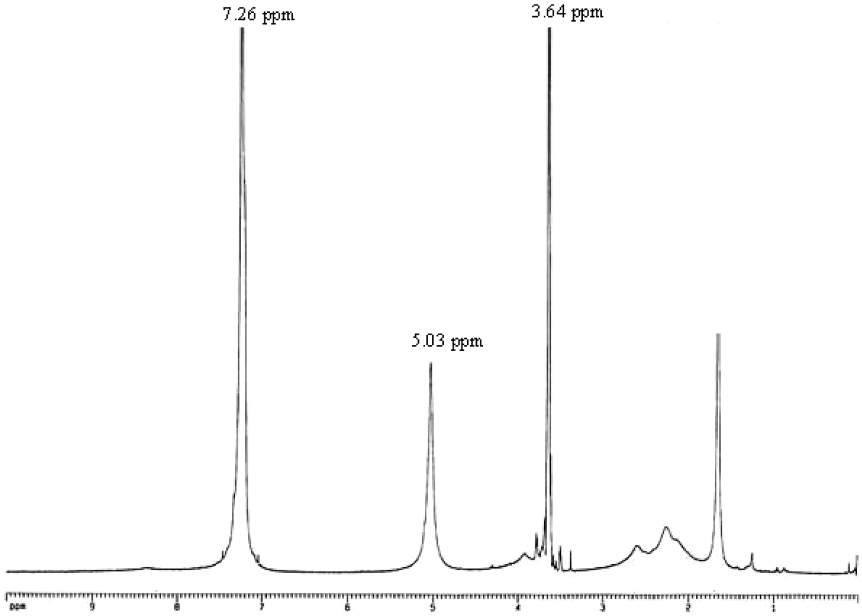
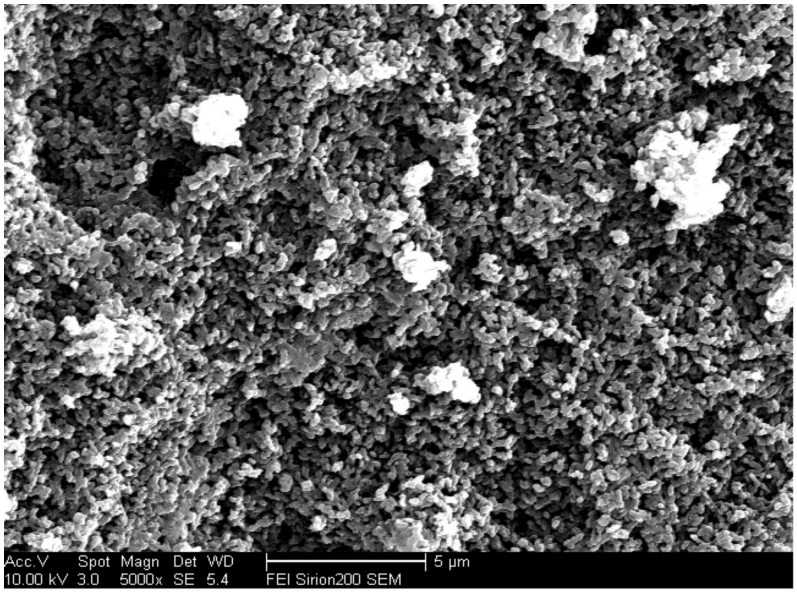


Fig. 1. ^{1}H-NMR spectrum of PBLG-*graft*-PEG1 in $CDCl_3$, where the grafting ratio of the copolymer is 26.2%.

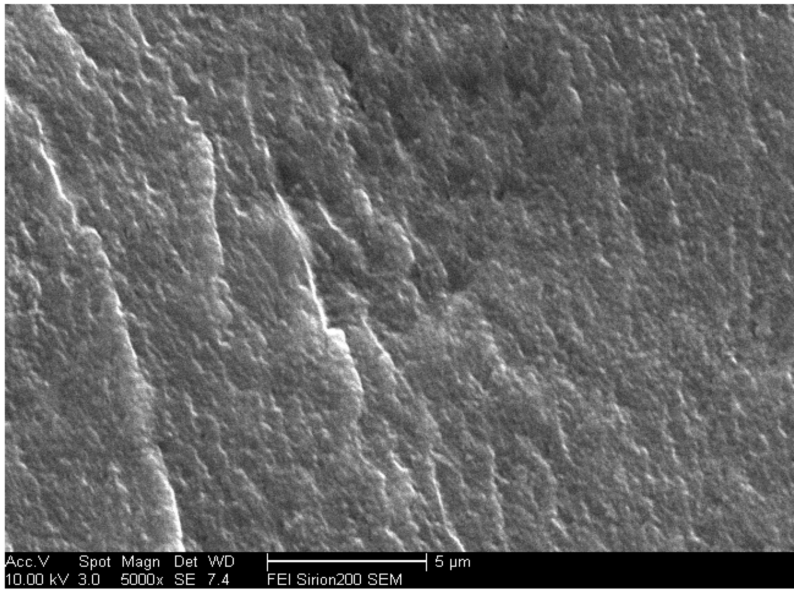
respectively) and the characteristic peak appearing at 3.64 ppm (corresponding to the ethylene protons of PEG segments) are detected (1, 19). This phenomenon demonstrates that PBLG-*graft*-PEG copolymers are composed of PBLG main-chains and PEG side-chains.

3.2 Morphological Studies

The morphologies of PBLG homopolymer and PBLG-*g*-PEG1 were evaluated by a scanning electron microscopy technique. Figures 2(a and b) show the photographs of a cross-section of PBLG homopolymer and PBLG-*g*-PEG1, respectively, where the grafting ratio of copolymer is 26.2%. As can be seen from Figure 2, the internal microstructure of PBLG homopolymer is much different from that of the PBLG-*g*-PEG1 copolymer. The microstructural image of PBLG homopolymer presents plenty of aggregates consisting of plentiful rice-like particles. As described in the document (21), polypeptide segments with hydrogen-bond take α-helix conformation in chloroform, suggesting the sample film cast from polypeptide solution in chloroform is still in α-helix conformation. This situation reveals that the



(a)



(b)

Fig. 2. SEM photographs of a cross-section of (a) PBLG and (b) PBLG-*graft*-PEG1, where the grafting ratio is 26.2% (magnification 5000×).

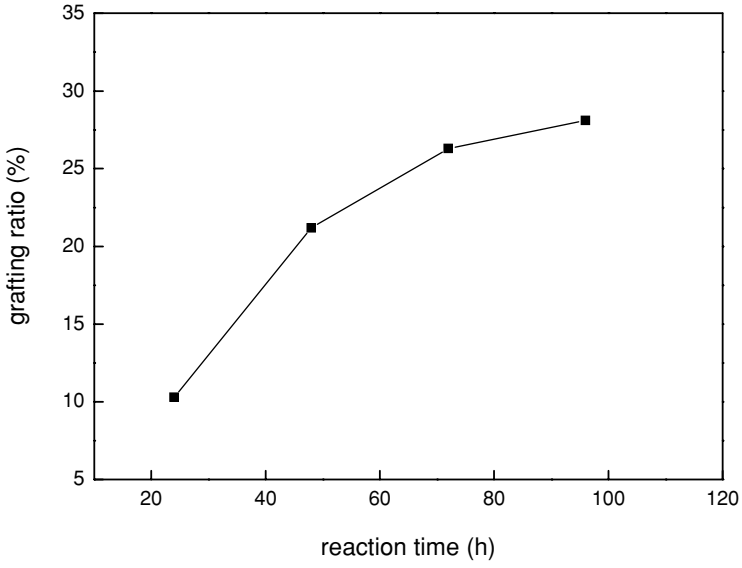


Fig. 3. The relationship between the reaction time and the grafting ratio of PBLG-*g*-PEG1, where the reaction temperature is 55°C.

microstructural morphology of PBLG homopolymer could be attributed to the α-helix structure of PBLG segments. The microstructural image of PBLG-*g*-PEG1 copolymer takes on a relatively rough surface bearing some micropores, which is caused by the interaction and better compatibility between the rigid PBLG main-chain and the soft PEG side-chain composed of (CH_2CH_2O) structural units. This situation verifies that the PEG chains have successfully been grafted onto the PBLG segments.

3.3 Effects of Reaction Time on the Grafting Ratio of Graft Copolymers

Figure 3 presents the relationship between the reaction time and the grafting ratio of PBLG-*g*-PEG1, where the reaction temperature is 55°C. As shown in Figure 3, the grafting ratio of copolymer increases with an increase in reaction time. As known, the ester exchange reaction is a reversible process, and the reaction degree depends on the reactants ratio and catalyst etc. At the start of reaction, mPEG is excessive, and the positive reaction is faster than the negative reaction. This situation reveals the increase of grafting ratio with increasing the reaction time. With the accumulation of resultants, the reaction speed gradually slows down. Accordingly, the increasing degree of grafting ratio gently debases.

3.4 Effects of Reaction Temperature on the Grafting Ratio of Graft Copolymer

Table 1 shows the effects of the reaction temperture on the grafting ratio of PBLG-*g*-PEG1, where the reaction time is 72 h. As seen from Table 1, the grafting ratio of copolymer increases with the elevation of reaction temperature. As mentioned above, the ester exchange reaction is a reversible process. For a reversible reaction, under the same reaction conditions, the elevation of reaction temperature promotes the positive reaction, as a result, the grafting ratio increases. In order to avoid the decomposing of polypeptide segments, the reaction temperature is usually controlled under 60°C.

3.5 Effects of The Chain Length of PEG on the Grafting Ratio of Graft Copolymer

Table 2 presents the effects of the chain length of PEG on the grafting ratio of graft copolymer, where the reaction tempearture is 55°C, the reaction time is 72 h. As can be seen from Table 2, the grafting ratio of copolymer decreases

Table 1. The effects of the reaction temperature on the grafting ratio of PBLG-*g*-PEG1*

Reaction temperature (°C)	*Grafting ratio (%)*
52	22.4
55	26.2
58	29.3

*Reaction time is 72 h.

Table 2. The effects of the chain length of PEG on the grafting ratio of graft copolymer*

Molecular weight of mPEG	*Grafting ratio (%)*
350	26.2
750	21.4
1300	13.1

*Reaction temperture is 55°C, the reaction time is 72 h.

with the increase of the chain length of mPEG. As noted, under the same reaction conditions, the longer the mPEG chain, the stronger the moving resistance, and the lower the reaction speed. This situation indicates the decrease of the grafting ratio with increasing the chain length of mPEG.

4 Conclusions

Poly(γ-benzyl-*L*-glutamate)-*graft*-poly(ethylene glycol) (PBLG-*graft*-PEG) copolymer has been synthesized by the ester exchange reaction of PBLG homopolymer with mPEG. NMR analysis proved that PBLG-*graft*-PEG copolymers were composed of PBLG main-chains and PEG side-chains. SEM photographs demonstrated that the grafting of the PEG chains onto the PBLG segments changed the microstructure of PBLG segments in copolymer. Experimental data testified that reaction temperature and time, and the chain length of PEG could change the grafting ratio of copolymer. Under the same reaction conditions, the grafting ratio of the copolymer increased with the increase of both reaction temperature and reaction time, while the augmentation of the chain length of PEG decreased the grafting ratio.

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