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Polymeric micelles formed by polypeptide graft copolymer and its mixtures with polypeptide block copolymer

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Abstract

Polymeric micelles based on poly(γ-benzyl-L-glutamate)–poly(ethylene glycol) graft copolymer (PBLG-*g*-PEG) with various degrees of grafting and the mixtures composed of PBLG-*g*-PEG and poly(γ-benzyl-L-glutamate)–poly(ethylene glycol) block copolymer (PBLG-*b*-PEG) were prepared by the dialysis method in deionized water. Fluorescence spectroscopy and transmission electron microscope (TEM) have been used to study the self-assembly behavior. The experimental results revealed that the degree of grafting exerts marked effect on the critical micelle concentration (CMC) and the morphology of the micelle formed by PBLG-*g*-PEG. With increasing the degree of grafting, the CMC value becomes larger and the morphology of formed micelle changes from irregular shape to spindle. It was also found that mixtures of PBLG-*g*-PEG/PBLG-*b*-PEG can associate into hybrid polymeric micelle with various shapes.

Keywords: Polypeptide copolymer; Self-assembly; Micelle

1. Introduction

In the past decade, chemists have shown great interest in constructing nanostructures with a variety of morphologies and functions via self-assembly because of their many potential applications as carriers of catalysts, enzymes, drugs, etc. [1–9]. Among the different approaches to self-assembly of macromolecules, micellization of block copolymers and graft copolymers in selective solvents is the most common one and has been investigated extensively [10–18].

Among the variety of self-assembled nanostructures, the polymeric micelles formed by graft copolymer and block copolymer composed of polypeptide segment and hydrophilic polymer chain have attracted attention for their great potential application such as drug delivery [19–28]. Cho et al. have studied polymer micelles composed of poly(γ-benzyl-L-glutamate) (PBLG) and poly(ethylene glycol) (PEG) block copolymer (PBLG-*b*-PEG) in aqueous media using TEM, fluorescence spectroscopy, and NMR. It was shown that PBLG–PEG block copolymer can form polymeric micelles with PBLG as the hydrophobic inner core and PEG as the hydrophilic shell. Critical micelle concentrations (CMC) of block copolymer, determined by the fluorescence measurements, decrease with increasing PBLG block length [20–26]. Recently, our group has studied the micelles based on polypeptide graft copolymer and its mixtures with polypeptide homopolymer. It was found that graft copolymer can associate to form polymeric micelles in the shape of spindle, which is different from the micelles formed by polypeptide block copolymer. The introduction of PBLG homopolymer into the PBLG-*g*-PEG system promotes the micellization [28].

However, to our knowledge, no experimental work on the study of the micelles of polypeptide graft copolymer with various degrees of grafting and the mixtures containing polypeptide graft and block copolymer has been reported so far. These related studies would contribute much to widening the research field of self-assembly. In the present contribution, the self-assembly behaviors of PBLG–PEG graft copolymer (PBLG-*g*-PEG) with various degrees of grafting and mixtures of PBLG-*g*-PEG and PBLG-*b*-PEG in aqueous media were investigated by fluorescent spectroscopy and TEM. With increasing the degree of grafting, the morphologies of micelles formed by PBLG-*g*-PEG transfer from irregular shape to spindle. Meanwhile, it was found that the mixtures of PBLG-*g*-PEG and PBLG-*b*-PEG can self-assembly into polymeric hybrid micelles in aqueous media.

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2. Experimental

2.1. Materials

Polyethylene glycol monomethylther (mPEG) (M_w=350) and methoxypolyethylene glycol amine (M_w=5000) were purchased from Sigma Inc., and used without further purification. Pyrene was also purchased from Sigma Inc., and used as received. Hexane, tetrahydrofuran (THF) and 1,4-dioxane are of analytical grade and dried with sodium to remove water before use. All other solvents are of analytical grade and used without further purification.

2.2. Syntheses of polypeptide copolymers

Poly(γ-benzyl-L-glutamate) was prepared by a standard *N*-carboxyanhydride (NCA) method [29,30]. PBLG was obtained by the ring-opening polymerization of *N*-carboxyl-γ-benzyl-L-glutamate anhydride (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 the PBLG sample was estimated to be 110,000 from the [η] value measured in dichloroacetic acid (DCA) according to the Doty et al. relation [31].

PBLG graft copolymers were prepared by the ester exchange reaction of PBLG with mPEG in 1,2-dichloroethane with *p*-toluenesulfonic acid as a catalyst according to the method described in our previous work [28]. The molecular weights of PBLG and mPEG used in the reaction are 110,000 and 350, respectively, and the PBLG/mPEG wt ratio is kept at 0.02. In all the cases, the mixture reacted at 55 °C for 1–3 days and then was precipitated into a large volume of anhydrous ethanol. The product was purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol, and then dried under vacuum. PBLG block copolymer was obtained by the ring-opening polymerization of γ-BLG NCA using methoxypolyethylene glycol amine (M_w=5000) as an initiator. The block and graft copolymer molecular weights and the degrees of grafting of the graft copolymers were estimated by nuclear magnetic resonance (NMR) measurements (Avance 550). It was calculated by the peak intensities of the methylene proton signal (5.1 ppm) of polypeptide and the ethylene proton signal (3.6 ppm) of PEG in the ^{1}H NMR spectrum. According to the NMR analysis, the molecular weight of the block copolymer is 65,000. The PBLG graft copolymers denoted as PBLG-*g*-PEG1, PBLG-*g*-PEG2, PBLG-*g*-PEG3, and PBLG-*g*-PEG4 have degrees of grafting of 20, 25, 31, and 36 mol%. The corresponding molecular weights are 134,000, 141,000, 148,000, and 154,000, respectively.

2.3. Preparation of micelles of polypeptide graft copolymer and its mixtures with polypeptide block copolymer

Micelles of PBLG-*g*-PEG and its mixture with PBLG-*b*-PEG were prepared by first dissolving PBLG-*g*-PEG and its mixture in a mixed solvent of tetrahydrofuran (THF)/*N*,*N*-dimethylformamide (DMF)[30/70, v/v] in a volumetric flask. The solution was then stirred at room temperature until the polymer was solubilized completely. The polymer concentration was maintained at 0.25 g/L. Next the solution was dialyzed against deionized water using cellulose dialysis tubing (type: Membra-cel, provided by Serva Electrophoresis GmbH, 3500 molecular weight cut-off) to form micelles and remove the organic solvents for about 48 h at room temperature. It was preferred that the deionized water was exchanged at intervals of 3–4 h. The obtained solution was diluted with deionized water to the desired concentration, then equilibrated at room temperature for 3–4 days before measurements. The polymer concentration for all the solutions refers to the weight percentage of the total copolymers in the water.

2.4. Measurements of fluorescence spectroscopy

To prove the formation of the micelles, fluorescence measurements were carried out using pyrene as a probe [32–35]. The fluorescence spectra of pyrene in aqueous solution were recorded at room temperature on a Varian Cary Eclipse fluorescence spectrophotometer. The concentration of pyrene in the final solution was 6×10^{-7} M. Before the fluorescence measurement, the solutions were heated for 3–4 h at 60 °C to equilibrate the pyrene and the micelles. Then the solutions were cooled down to room temperature, and equilibrated for 3–4 days before measurements. The emission wavelength was 390 nm for excitation spectra, and excitation bandwidth was 5 nm. All fluorescence spectra were measured at 25 °C.

2.5. Observation of transmission electron microscope

The morphology of the micelles was examined by TEM (JEM-1200-EXII). Drops of micelle solution were placed on a carbon film coated copper grid, and then were dried at room temperature. Before the observations, the sample was stained by phosphotungstic acid water solution (1.0 wt%). The TEM bright filed imaging was performed with 120 kV accelerating voltage.

3. Results and discussion

3.1. Formation of micelles

Pyrene has been widely used as a probe to monitor the association and micellization of macromolecules in solutions because its photophysical character changes with the variation of the existing environment [32–35]. Fluorescence excitation spectra obtained for pyrene in solutions of PBLG-*g*-PEG showed that the fluorescence intensity increases with

increasing polymer concentration. Concomitantly with the increase in the fluorescence intensity, a red shift of the pyrene (0, 0) band from 333 to 338 nm is shown, indicating that micellization takes place for the PBLG–PEG graft copolymer. Such results can be attributed to the transfer of pyrene molecules from water to a hydrophobic environment within the micelles core. Similar phenomena were also observed for the mixed systems of PBLG–PEG graft and block copolymers. A red shift and the enhanced fluorescence intensity are observed, suggesting that the micellization occurs.

Curve (a) in Fig. 1 illustrates a typical plot of I_{338}/I_{333} versus LgC for PBLG–PEG graft copolymer. As it can be seen from Fig. 1, negligible changes in the magnitude of I_{338}/I_{333} were observed at low concentrations. As the polymer content increases, at a certain concentration (i.e. the CMC) the value of I_{338}/I_{333} increases dramatically in a sigmoid manner. According to the method adopted by Winnik et al. [32], the CMC value is taken as the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low polymer concentrations. The CMC value for PBLG-*g*-PEG2 is obtained at the value 2.0×10^{-3} g/L. Curve (b) shows a typical plot of I_{338}/I_{333} versus LgC for the mixed system (PBLG-*g*-PEG4/PBLG-*b*-PEG = 80/20 in weight). Similar to the results of the micellization of PBLG graft copolymer, the ratio exhibits a flat region in the low concentration extreme and increases abruptly in a sigmoid manner at a certain concentration, indicating the onset of aggregation. The obtained CMC value is 2.2×10^{-3} g/L.

The CMC values for PBLG-*g*-PEG with various degrees of grafting and the mixtures composed of PBLG-*g*-PEG and PBLG-*b*-PEG determined by fluorescence spectroscopy measurements are listed in Table 1. As it can be seen, the CMC value for the PBLG–PEG graft copolymer becomes larger with increasing the degree of grafting, suggesting that the self-assembly tendency of PBLG graft copolymer decreases. It can be explained that with increasing the degree of grafting, the EO mol content increases from 0.62 to 0.74 as it can be seen from Table 1. As a result, the hydrophobic property of the copolymer gets weaker, leading to a lower aggregating force in water [36,37]. With respects to the micellization of mixtures of PBLG–PEG graft and block copolymers, shown in Table 1, the apparent CMC values are between those of individual PBLG copolymers. This is in accord with the fact

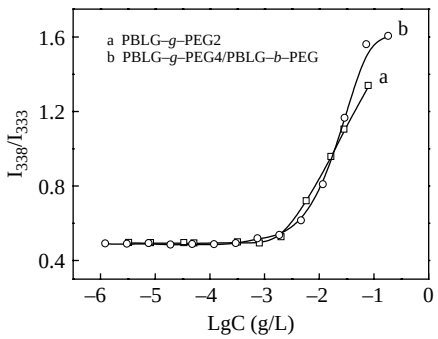

Fig. 1. Plots of I_{338}/I_{333} vs LgC for PBLG-*g*-PEG2 and mixtures of PBLG-*g*-PEG4/PBLG-*b*-PEG with a weight ratio of 80/20 in deionized water.

Table 1
CMC values of PBLG-*g*-PEG with various degrees of grafting and the mixtures composed of PBLG-*g*-PEG4 and PBLG-*b*-PEG with various weight ratios determined by fluorescence spectroscopy measurements

Sample	PBLG-*g*-PEG/ PBLG-*b*-PEG (wt/wt)	EO content (mol%)	CMC ($\times10^{-3}$ g/L)
PBLG-*g*-PEG1	100/0	0.62	1.6
PBLG-*g*-PEG2	100/0	0.67	2.0
PBLG-*g*-PEG3	100/0	0.71	2.2
PBLG-*g*-PEG4	100/0	0.74	2.4
PBLG-*g*-PEG4/ PBLG-*b*-PEG	80/20	0.58	2.2
PBLG-*g*-PEG4/ PBLG-*b*-PEG	50/50	0.43	2.1
PBLG-*g*-PEG4/ PBLG-*b*-PEG	20/80	0.34	2.0
PBLG-*g*-PEG4/ PBLG-*b*-PEG	10/90	0.31	1.8
PBLG-*g*-PEG4/ PBLG-*b*-PEG	0/100	0.29	1.7

that the EO contents or polypeptide contents (the polypeptide content in the mixture is associated with the driving force for the micellization) for the mixtures are between those of PBLG graft and block copolymers (Table 1). For the mixed systems, only one CMC point was observed, indicating that PBLG–PEG graft and block copolymers may take part in the hybridization together and form hybrid micelle. One CMC point was also observed for self-association of mixed amphiphilic copolymers by Chu et al. [38]. In their work, two oxyalkylene triblock copolymers can self-associate to form hybrid micelles in aqueous solution, and one CMC point exhibits.

3.2. Observations of micelle morphologies

Fig. 2(a)–(d) displays the morphologies of micelles formed by PBLG–PEG graft copolymer with various degrees of grafting observed by TEM. Micelles with irregular shapes are shown in Fig. 2(a) for PBLG-*g*-PEG1 with 20% degree of grafting. As the degree of grafting increases to 25%, an apparent variation in morphology of PBLG-*g*-PEG2 micelle takes place. Irregular shape turns to spherical shape, as shown in Fig. 2(b). Further, increasing the degree of grafting to 31%, the morphology of micelle formed by PBLG-*g*-PEG3 is riziform shape (Fig. 2(c)). Finally, as the degree of grafting increases to 36%, the micelle based on PBLG-*g*-PEG4 is characteristic of spindly shape (Fig. 2(d)).

Shown in Fig. 2(e)–(i) are the images of micelles formed by the mixtures PBLG-*g*-PEG4/PBLG-*b*-PEG with various weight ratios (80/20, 50/50, 20/80, 10/90, 0/100), respectively. Fig. 2(i) shows the typical spherical shapes of micelles based on PBLG-*b*-PEG. The morphologies have a significant change as the weight ratios of PBLG-*g*-PEG4 to PBLG-*b*-PEG vary. Shown in Fig. 2(e) is the morphology of the micelle formed by the mixture with 80 wt% PBLG-*g*-PEG4 content. Although, the micelles exhibit spindle shape, changes in the morphologies take place. Some micelles tend to be cylindrical. At the 50 and 20 wt% content of

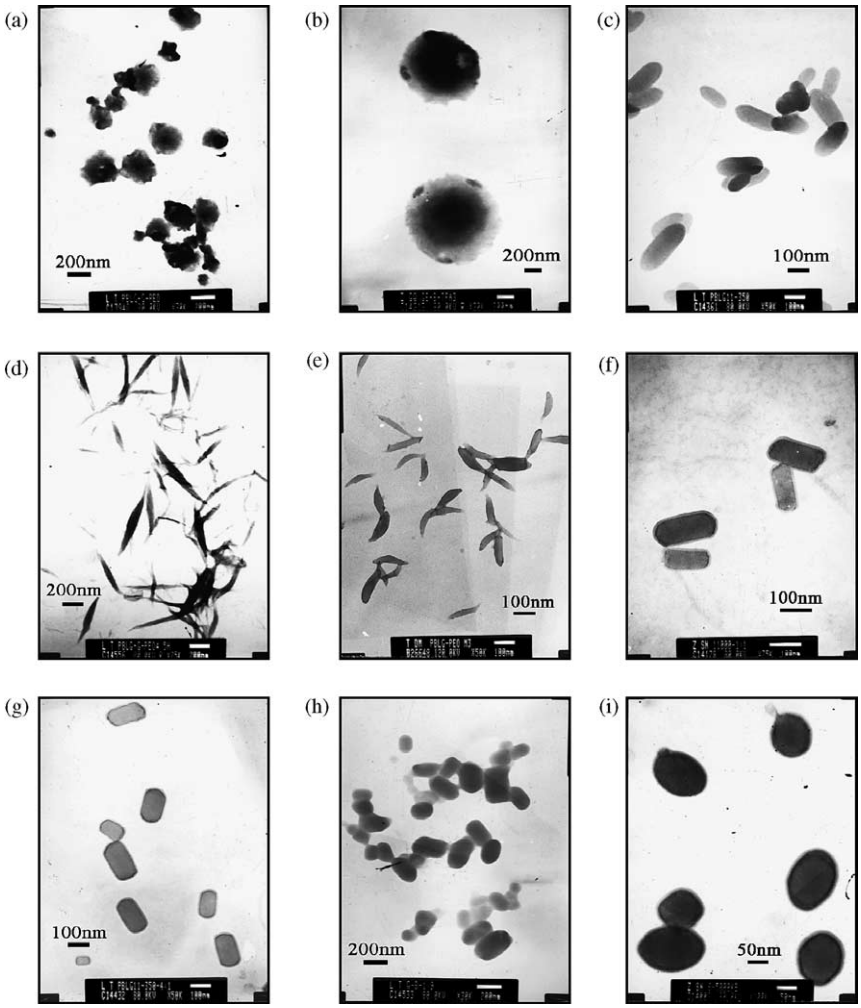

Fig. 2. TEM photographs of micelles formed by PBLG-*g*-PEG with degrees of grafting of 20% (a) 25% (b) 31% (c) 36% (d) and mixtures of PBLG-*g*-PEG4/PBLG-*b*-PEG with weight ratios of 80/20 (e) 50/50 (f) 20/80 (g) 10/90 (h) 0/100 (i).

PBLG-*g*-PEG4, as shown in Fig. 2(f) and (g), the micelles almost have the cylindrical shapes. As the PBLG-*b*-PEG content reaches 90 wt%, most micelles are cylindrical shape, while a small part of the micelles turns to be spherical (Fig. 2(h)). Accumulation of the obtained results suggests that PBLG–PEG graft copolymer can hybrid with PBLG–PEG block copolymer and leading to formation of the polymeric hybrid micelle, different from the micelles formed by the individual PBLG graft and block copolymer. In the process of micellization, a small part of PBLG-*g*-PEG and PBLG-*b*-PEG may form its own micelle independently, especially as one component is very higher in the mixed system. It could be due to that there is one optimized composition for two copolymers to form hybrid micelles. Within the optimized composition range, PBLG-*g*-PEG and PBLG-*b*-PEG can form the hybrid micelles. When the composition is out of the optimized range, besides the hybrid micelles, the exceed part can form its own micelles.

In summary, the micellization behaviors of PBLG-*g*-PEG with various degrees of grafting and the mixtures with PBLG-*b*-PEG in various weight ratios in aqueous media were studied by fluorescence spectroscopy and TEM. The experimental results revealed that PBLG-*g*-PEG and its mixtures can self-associate into polymeric micelles in aqueous media. The morphologies of PBLG-*g*-PEG micelles change markedly with increase of the degree of grafting. The CMC values become larger with increasing the degree of grafting, suggesting that it forms micelles more difficultly. Meanwhile, PBLG–PEG graft and block copolymers can take part in the comicellization and form polymeric hybrid micelle. The morphologies of micelles formed by PBLG-*g*-PEG and PBLG-*b*-PEG mixtures vary from spindle to sphere as the PBLG-*b*-PEG content changes from 0 to 100 wt%.

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