Polymer 55 (2014) 602–610

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Self-assembly behavior of rod–coil–rod polypeptide block copolymers

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ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form
2 December 2013
Accepted 7 December 2013
Available online 14 December 2013

Keywords:
Polypeptide
Self-assembly
Rod–coil block copolymer

ABSTRACT

Self-assembly behavior of rod–coil–rod poly(γ-benzyl-L-glutamate)-*b*-poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate) (PBLG-*b*-PEG-*b*-PBLG) triblock copolymers with various PBLG block lengths in aqueous solution was investigated. The PBLG-*b*-PEG-*b*-PBLG triblock copolymers are able to self-assemble into vesicles when PBLG block length is relatively short. Meanwhile, the initial polymer concentration was found to have influence on the self-assembly. Giant vesicles can be observed when the initial concentration is high. Dissipative particle dynamics (DPD) simulations about the vesicles revealed that the rigid rod blocks could be aligned parallelly with each other to form the monolayer vesicles wall. When the PBLG block length in the PBLG-*b*-PEG-*b*-PBLG triblock copolymers increases, the aggregate morphologies were observed to transform from vesicles to spherical micelles. Based on the experimental and simulation results, we proposed a possible mechanism of the morphological transitions of the rod–coil–rod triblock copolymer aggregates.

1. Introduction

Amphiphilic block copolymers are able to self-assemble into various supramolecular aggregates in selective solvents. Diversiform morphologies such as spheres, rods, vesicles, spindles, tubules, toroids, and other complex structures have been observed [1–5]. These structures have attracted widespread interest for their potential applications in drug delivery systems, coatings, cosmetics, and nanoreactors [6–9]. In the past decade, many studies have been carried out on the self-assembly of coil–coil type amphiphilic block copolymers. For example, in a series of literature, Eisenberg et al. reported the self-assembly behavior of amphiphilic polystyrene-based block copolymers (*e.g.*, polystyrene-*b*-poly(acrylic acid) and polystyrene-*b*-poly(ethylene oxide)). They revealed that by adjusting the external conditions such as solvents, block length, pH value, and salt addition, the morphologies of aggregates can be well manipulated [10–12].

Rod–coil block copolymers, which consist of a rigid rod segment jointed to a flexible coil block, exhibit much more distinct self-assembly features because of the ordered packing mode of rigid blocks during aggregation and the additional interactions that can occur between anisotropic rod blocks [13–18]. Thus, rod–coil block copolymers are conceived to possibly have different self-assembly behavior from coil–coil ones [19–23]. Jenekhe's group reported that poly(phenylquinoline)-*b*-polystyrene (PPQ-*b*-PS) rod–coil block copolymers can self-assemble into diverse aggregates in organic solvents by tuning the PPQ rod block length or solvent quality. Among those aggregates, the vesicles have a specific single-layer structure owing to the dense and ordered packing of the π-conjugated PPQ rod blocks [22,23]. Self-assembly of these rod–coil block copolymers provides an opportunity to generate functional materials with well-defined supramolecular architectures and special properties. So far, self-assembly of rod–coil block copolymers with short rod block has been focused [13,24]. However, due to the limitation of synthesis technology and poor solubility of rod blocks in solvents, little attention was devoted to the self-assembly of rod-coil block copolymers with long rigid rod [14,19].

Recently, increasing attention has been paid to the polypeptide-based self-assemblies due to their biocompatibility and advantages in controlling both the functions and structures of the supramolecular aggregates [25–29]. Polypeptides can adopt well ordered secondary structure such as α-helix and β-sheet, as a result of the intra- and inter-molecular hydrogen bonds, respectively [29,30]. The α-helical structure is thought to give rise to a rigid-rod structure, which can be employed as a model rigid-rod polymeric system. The incorporation of polypeptide-based rigid chains and other synthetic coil chains is a particular valuable approach to creating novel supramolecular architectures [31–36]. For example, Chang

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0032-3861/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymer.2013.12.016

et al. studied the self-assembly behavior of poly(Z-L-lysine)-*block*-poly(*N*-isopropylacrylamide) (PZLys-*b*-PNIPAm) rod–coil block copolymers and found that these amphiphilic block copolymers are able to form vesicles in dilute solution, in which the rigid polypeptide chains take side by side packing mode to form the vesicle wall [35]. Our group reported the aggregation behavior of PBLG-*b*-PEG block copolymers in chloroform/ethanol/trifluoroacetic acid (TFA) mixed solution [36]. The micelles are formed with PEG as the shell and PBLG as the core. In the absence of TFA, PBLG adopts a rigid α-helix conformation, and the block copolymers self-assemble into cylindrical micelles. The polypeptide blocks within the cores are interdigitated and favor ordered parallel packing, with their long axis aligning in an orientation vector. The vector could gradually change along the long-center-axis of the micelle in a cholesteric liquid crystal manner. When the denaturant acid TFA is added, polypeptide chains become random coils. Spherical micelles with coiled polypeptide blocks randomly packing inside the cores are formed.

Generally, self-assembly of polypeptide-based rod–coil block copolymers with short rod block (or low volume fraction of rod block) has been well studied, and universal aggregate morphologies such as spherical micelles, cylindrical micelles, and vesicles were observed [29,31,35–39]. However, limited attention was focused on the self-assembly of the polypeptide block copolymers with long rod block (or high volume fraction of rod block). The underlying complex mechanism of the self-assembly of polypeptide-based copolymers with various lengths of polypeptide chain is still not clear enough.

In this work, we report the self-assembly behavior of rod–coil–rod poly(γ-benzyl-L-glutamate)-*b*-poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate) (PBLG-*b*-PEG-*b*-PBLG) triblock copolymers with various rigid PBLG block lengths. It was found that the PBLG-*b*-PEG-*b*-PBLG triblock copolymers containing relatively short PBLG block length can self-assemble into vesicles, and giant vesicles can be obtained at high initial concentrations. Moreover, dissipative particle dynamics (DPD) simulations about the vesicles revealed that the rigid rod blocks are aligned parallelly with each other to form the monolayer vesicle wall. Interestingly, with increasing the PBLG block length, the aggregate morphologies transform from vesicles to spherical micelles. By combining experimental findings with DPD results, a possible mechanism of the morphological transitions of the rod–coil–rod triblock copolymer aggregates is suggested.

2. Experimental section

2.1. Materials

α,ω-Diamino-poly(ethylene glycol) (NH_2–PEG–NH_2) (M_w = 2000) was purchased from Sigma Inc. Analytical grade of hexane, tetrahydrofuran (THF), and 1,4-dioxane were refluxed with sodium and distilled immediately before use. All the other reagents are of analytical grade and used as received. The dialysis bag (Membra-cel, 3500 molecular weight cutoff) was provided by Serva Electrophoresis GmbH.

2.2. Synthesis of PBLG-b-PEG-b-PBLG triblock copolymers

Poly(γ-benzyl-L-glutamate)-*block*-poly(ethylene glycol)-*block*-poly(γ-benzyl-L-glutamate) triblock copolymers (PBLG-*b*-PEG-*b*-PBLG, abbreviated as BEB) were synthesized by ring-opening polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride (BLG-NCA) initiated by NH_2–PEG–NH_2 with 1,4-dioxane as solvent [40,41]. After reacted at room temperature for 3 days, the viscous 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. Finally, the product was dried under vacuum and white power was collected.

2.3. Synthesis of P(BLG-co-ELG)-b-PEG-b-P(BLG-co-ELG) block-random copolymers

Poly((γ-benzyl-*co*-γ-ethyl)-L-glutamate)-*block*-poly(ethylene glycol)-*block*-poly((γ-benzyl-*co*-γ-ethyl)-L-glutamate) block-random copolymers (P(BLG-*co*-ELG)-*b*-PEG-*b*-P(BLG-*co*-ELG), abbreviated as BEB-Et) were prepared by ester exchange reaction of BEB triblock copolymer with ethanol [42,43]. The reaction was performed at 55 °C in 1,2-dichloroethane (DCE) with *p*-toluenesfulfuric acid (TSA) as a catalyst. Then the reaction mixture was precipitated into a large volume of anhydrous methanol. The product was purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol and then dried under vacuum. By the variation of molar ratio of BLG unit to ethanol and the reaction time, the degree of substitution of ethyl group (ratio of number of ELG unit to total number of BLG and ELG units) can be adjusted.

2.4. Preparation of micelles

The polymeric micelle solutions were prepared by using a dialysis method. First, the BEB triblock copolymers were dissolved in THF by stirring at room temperature for 2 days to obtain stock solutions with various initial polymer concentrations. To prepare micelle solution, 5 mL of deionized water, a selective solvent for PEG, was added to 10 mL of polymer solution at a rate of 0.01 mL/s with vigorous stirring. Subsequently, all the solution was dialyzed against deionized water for 3 days to ensure that all the organic solvents were removed. Before analysis, the solutions were stabilized for at least 5 days.

2.5. ^1H NMR

The composition of all copolymers was determined by ^1H NMR spectrum (Avance 550, Bruker) using deuterated chloroform ($CDCl_3$) with 15 vol% deuterated trifluoroacetic acid (TFA-d) as solvent and tetramethylsilane (TMS) as an internal standard.

2.6. Gel permeation chromatography

The polydispersity index of BEB triblock copolymers was determined by gel permeation chromatography (GPC, Varian, PL GPC-50 plus). A 20 mM LiBr/DMF solution was used as the mobile phase at the flow rate of 0.8 mL/min (T = 49 °C). The calibration curve was obtained by narrow polydispersity PS standards.

2.7. Circular dichroism (CD)

CD analyses of the polypeptides were performed with a Chirascan spectrometer at room temperature. The diluted solutions (0.01 mg/mL) were introduced in quartz cells with 1 cm optical path length. Wavelengths between 190 and 300 nm were analyzed, with an integration time of 0.5 s and a wavelength step of 1 nm.

2.8. Fourier transform infrared spectrum (FTIR)

FTIR spectra of the samples were recorded on a Nicolet 5700 FTIR spectrometer at frequencies ranging from 400 to 4000 cm^{-1}. The sample powers were thoroughly mixed with KBr and pressed into pellet form. The tests were performed at room temperature.