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Aggregate structure change induced by intramolecular helix–coil transition

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Abstract

Self-assembly behavior of poly(γ-benzyl L-glutamate)-*graft*-poly(ethylene glycol) (PBLG-*g*-PEG) in ethanol medium was studied by transmission electron microscopy (TEM), scanning electron microscopy (SEM), laser light scattering (LLS), and circular dichroism (CD). The experimental results revealed that the conformation change of the polypeptide graft copolymer exerts marked effect on its self-association behavior. Spindle-like micelles with polypeptide blocks aligned inside the cores are formed in ethanol solution without denaturant acid. When the denaturant acid is added, the rigid α-helix transforms to random coil, resulting in an aggregate structure change from the spindle-like micelle to large compound micelle. For the large compound micelles, the coiled polypeptide chains and PEG blocks pack randomly within the cores, surrounded by the PEG chains outside to stabilize the aggregates.

Keywords: Polypeptide copolymer; Self-assembly; Helix–coil transition

1. Introduction

Recently, supramolecular assemblies formed through the association of macromolecules receive considerable attention. Particularly, many intensive studies have been carried out on the association of copolymers in selective solvents for the formation of polymeric micelles with a core–shell structure [1–4]. The micelles formed by the block or graft copolymers are characterized by their small size and high stability, leading to promising applications in fields such as drug delivery, catalysis, and nanoreactors etc.[5–9].

Among various copolymers, polypeptide copolymer receives attention for self-assembly and biopolymeric characteristics. Micelles with various shapes were observed for polypeptide copolymers with different molecular structures [10–17]. For example, Rodríguez-Hernández et al. reported formation of schizophrenic vesicles based on a zwitterionic diblock copolymer poly(L-glutamic acid)-*block*-poly(L-lysine) [12]. The hydrophobicities of the two polypeptide blocks vary with the changes in the pH value in aqueous medium, leading to formation of vesicles with different supramolecular structures. Cho et al. studied polymeric micelles composed of poly(γ-benzyl L-glutamate) (PBLG) and poly(ethylene glycol) (PEG) block copolymer (PBLG-*b*-PEG) in aqueous medium. It was found that PBLG-*b*-PEG block copolymer could form spherical micelles with PBLG as the hydrophobic inner core and PEG as the hydrophilic shell. Critical micelle concentration (CMC) of the block copolymer, determined by the fluorescence measurements, decreases with increasing PBLG block length [13].

It is well known that polypeptides show various conformations, such as α-helix, and random coil [18–20]. The conformation adopted by the polypeptides depends on solvent, temperature, etc. For example, PBLG takes α-helix form when it is dissolved in neutral organic solvent. The α-helix can transform to the

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random coil as denaturant organic acid is introduced. The polypeptide conformation has been shown to have a significant effect on its aggregate behaviors such as liquid crystalline ordering. Polypeptides such as PBLG with rigid α-helix conformation can form liquid crystalline structure, where chains are orderly aligned, above a critical polymer concentration in numerous organic solvents. When a denaturant organic acid such as trifluoroacetic acid (TFA) is added, α-helix to coil transition takes place due to the disruption of helix structure. The polypeptide chain becomes flexible and unable to support the orientational ordering, and an anisotropic to isotropic phase transition occurs accordingly [21–25]. With respect to the relation between the chain conformation and self-assembly behavior of the polypeptides, the studies are limited. The related studies would be helpful for a more comprehensive understanding of the self-association behavior of the polypeptides.

In this communication, the effect of the conformation change of poly(γ-benzyl L-glutamate)-*graft*-poly(ethylene glycol) (PBLG-*g*-PEG) on its self-assembly behavior is reported. The experimental results revealed that the aggregates formed by the PBLG-*g*-PEG transforms from spindle-like micelle to large compound micelle with the change in the conformation of PBLG chain from α-helix to random coil. Based on the information gained through the experiments, the mechanism regarding the effect of conformation change of the polypeptide graft copolymer on its self-association behavior is suggested.

2. Experimental

Polyethylene glycol monomethylether (mPEG) ($M_w = 350$) was purchased from Sigma Inc., and used without further purification. 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.

Poly(γ-benzyl L-glutamate) was prepared by a standard *N*-carboxyanhydride (NCA) method [21,24]. 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 120,000 from the $[\eta]$ value measured in dichloroacetic acid (DCA) [21].

PBLG graft copolymer was 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 [24]. The molecular weights of PBLG and mPEG used in the reaction are 120,000 and 350, respectively and the PBLG/mPEG wt ratio is kept at 0.02. The mixture was reacted at 55 °C for 3 days and then 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. The degree of grafting of the graft copolymer was estimated by nuclear magnetic resonance (NMR) measurements (Avance 550, BRUKER). 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. Calculation, according to the NMR analysis, showed that the degree of grafting for the PBLG-*g*-PEG is 25.1 mol%.

The obtained PBLG-*g*-PEG sample was first dissolved in $CHCl_3$/TFA mixed solvents with various TFA contents. The polymer concentration of the initial solution was maintained at 0.45 g/L. Then ethanol, as a precipitant, was added at a rate of 1 drop every 8–10 s with vigorous stirring. In all the cases, the ethanol was added until the polymer concentration became 0.09 g/L.

The morphologies of the micelles were examined by TEM (JEM-1200-EXII, JEOL). 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 aqueous phosphotungstic acid solution (1.0 wt%). The TEM bright field imaging was performed with 120 kV accelerating voltage.

The SEM observations were conducted on a JSM-6460 (JEOL) electron microscope at an accelerating voltage of 20 kV. The specimens were prepared by depositing a drop of the micelle solution onto a glass slide, and coated with carbon before SEM observations.

Light scattering was measured by a commercial LLS spectrometer (CGS-5022, ALV) equipped with an ALV-High QE APD detector and an ALV-5000 digital correlator using a He–Ne laser (the wavelength $\lambda = 633$ nm) as light source. All the measurements were carried out at 25 °C. In static LLS, the angular dependence of the excess absolute time-average scattered intensity, i.e., Rayleigh ratio $R_{vv}(q)$ of a dilute dispersion leads to the root-mean square *z*-average radius of gyration $\langle R_g^2 \rangle^{1/2}$ (or simply $\langle R_g \rangle$), where $q = 4\pi n \sin(\theta/2)/\lambda$ is the scattering vector as a function of scattering angle θ, n is the refractive index of the solution, and λ is the wavelength of the incident beam. In dynamic LLS, the Laplace inversion of a measured intensity–intensity time correlation function $G^{(2)}(t,q)$ in the self-beating mode could result in a line-width distribution $G(\Gamma)$. The translational diffusion coefficient D calculated from the decay rate, Γ, by the slope of the Γ vs. q^2 plot, can lead to hydrodynamic radius R_h by the Stokes–Einstein equation $R_h = k_B T/(6\pi\eta D)$, where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.

Circular dichroism analyses of the conformation of polypeptide blocks were performed with a JASCO J-810 spectrometer. The micelle solutions were introduced in quartz cells with 0.5 mm optical path length and measured at room temperature.

3. Results and discussion

Initial polymer solutions for self-assembly of PBLG-*g*-PEG were prepared by dissolving the PBLG-*g*-PEG samples in $CHCl_3$/TFA mixed solvents with various acid contents. Self-assembly occurred as ethanol was added to desolvate the insoluble polypeptide blocks from solution. Fig. 1 displays the morphologies of the PBLG-*g*-PEG aggregates formed in the

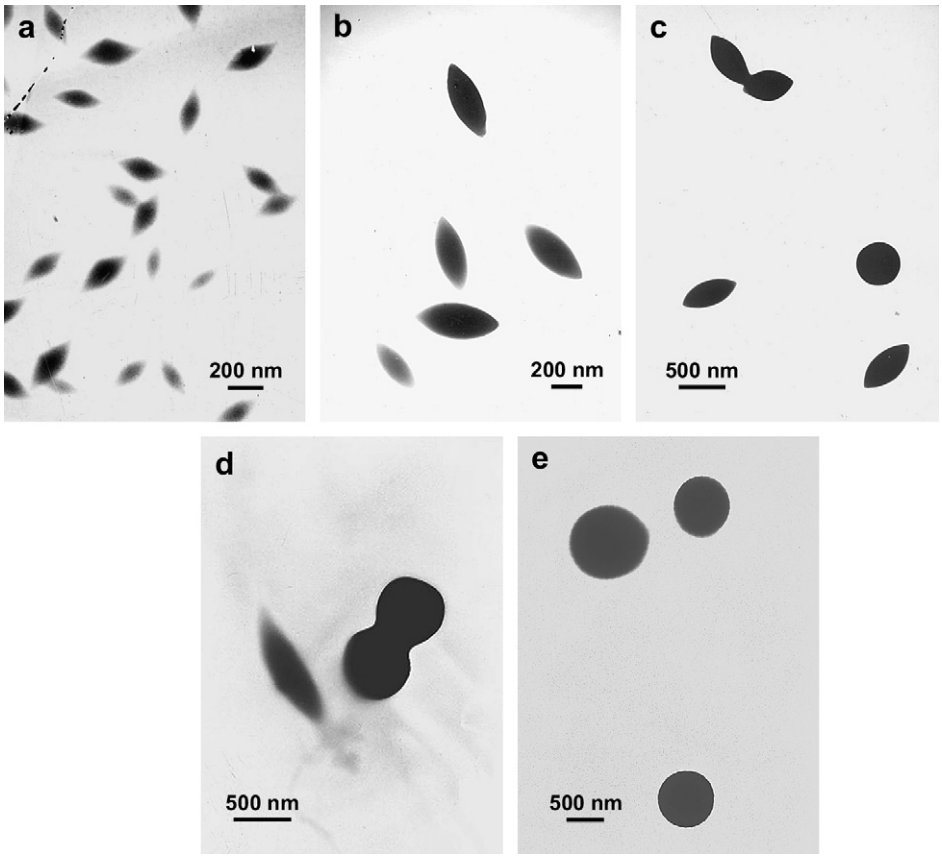


Fig. 1. TEM photographs of the PBLG-*g*-PEG aggregates formed in ethanol solutions with TFA mole fractions of 0 (a), 0.004 (b), 0.012 (c), 0.016 (d), and 0.032 (e).

ethanol solutions with various TFA mole fractions. For the solutions with TFA mole fractions of 0 and 0.004, micelles with regular spindle shape were found as shown in Fig. 1(a) and (b). When the TFA mole fraction increases to 0.012 and 0.016, an apparent change in the morphologies of the formed aggregates takes place. Some spindle micelles turn to be spherical as shown in Fig. 1(c) and (d). With further increase in TFA mole fraction, all the aggregates become spherical. A typical example is given in Fig. 1(e). It is also noted that concomitantly with the changes in the morphologies, the micelle size turns larger substantially.

The morphologies of the aggregates formed by the PBLG-*g*-PEG sample and also their changes upon adding the denaturant acid are also provided by the SEM observations. Fig. 2(a) shows the aggregate morphology of the PBLG-*g*-PEG in the neutral solution. Spindle-like aggregates are observed, which are in line with the TEM results. When the TFA is added, the aggregates turn spherical and larger as shown in Fig. 2(b). Further increasing the acid content gives rise to large spherical aggregates (Fig. 2(c)).

Self-assembly of PBLG-*g*-PEG was further studied by laser light scattering (LLS). Fig. 3 shows typical hydrodynamic radius $\langle R_h \rangle$ distributions for aggregates in the ethanol solutions with various TFA mole fractions. Narrow distributions of the particle size were found for all the samples examined. With changing the TFA mole fraction from 0 to 0.032, the particle size, as indicated by the $\langle R_h \rangle$ value, tends to be larger. Such a result is in reasonable agreement with the TEM and SEM observations, i.e., the aggregate size increases with increasing acid content in the solution. The influence of denaturant acid content on the aggregate size can be further viewed in the inset plot where $\langle R_h \rangle$ is plotted against TFA content. The $\langle R_h \rangle$ is 197 nm for the micelles in the solution without TFA, and it increases with increasing acid content. At higher acid contents, the acid effect on the aggregate size becomes less pronounced.

The chain conformation of the polypeptide block within the aggregate cores was examined by circular dichroism (CD), which is an effective way to determine the polypeptide conformation [26–28]. Fig. 4 demonstrates the CD spectra for the micelle solutions with various TFA mole fractions. Due to the intense absorption originating from the solvent ($CHCl_3$) [26], the CD spectra were not collected at shorter wavelength. The spectrum obtained for aggregate solution without TFA shows a minimum at 233 nm, suggesting that the polypeptide adopts α-helical structure [26–28]. It is noted that the wavelength of this minimum peak is longer than the typical value of 222 nm reported for PBLG [26–28]. This band is widely reported to be assigned to $n-\pi^*$ transition of peptide bonds, which can be influenced by many factors such as solvent, side chain, and intramolecular or intermolecular interactions [29,30]. In the present work, the red-shifted band in CD spectra could be attributed to the aggregation of the PBLG blocks within the aggregate core. Such an aggregation may change the environment that surrounds the PBLG chains, resulting

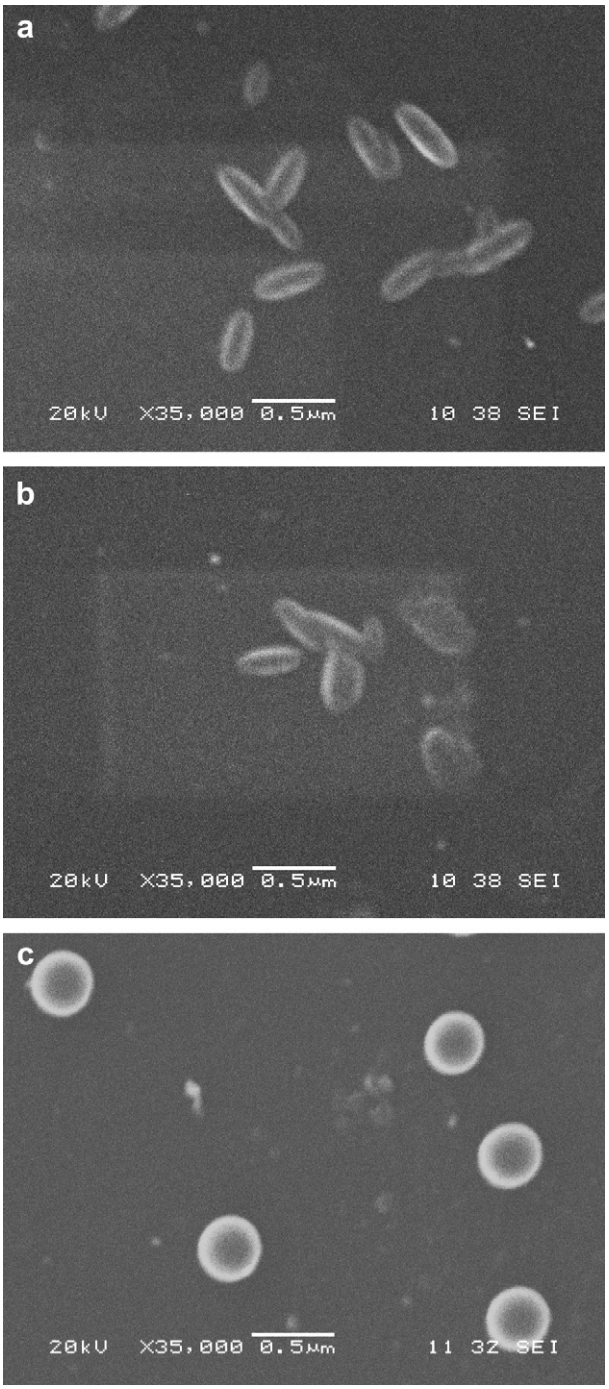


Fig. 2. SEM photographs of the PBLG-*g*-PEG aggregates formed in ethanol solutions with TFA mole fractions of 0 (a), 0.009 (b), and 0.029 (c).

in a peak position shift [13]. When the acid content is increased, the magnitude of the minimum peak tends to diminish, indicating the α-helix gradually transforms to the random coil. The complete extinction of the α-helix band at high acid content implies that the polypeptide blocks take random coil conformation.

Based on the information gained through the experiments, the mechanism regarding the effect of conformation change of the polypeptide graft copolymer on its self-assembly is proposed as follows. Shown in Scheme 1 is a schematic

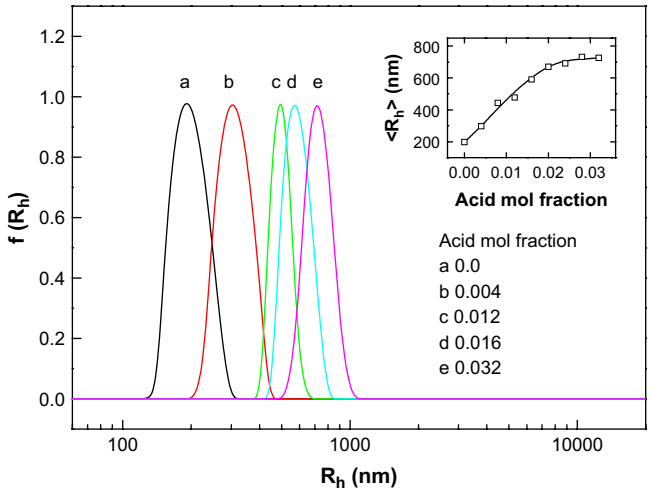


Fig. 3. Typical hydrodynamic radius distributions $f(R_h)$ of the PBLG-*g*-PEG aggregates formed in ethanol solutions with various TFA mole fractions. Shown in inset is plot of $\langle R_h \rangle$ versus denaturant acid concentration.

illustration of PBLG-*g*-PEG aggregates formed at different conditions. As evidenced by the CD spectra, the polypeptide blocks within the aggregate cores take helix conformation in the solution without TFA. Due to the rigid helix chain structure, the polypeptide blocks within the cores are expected to favor ordered packing with their long axes aligned in a nematic liquid crystal manner. The flexible PEG chains could be excluded out of the polypeptide aggregate core to form a shell, stabling the formed aggregates. The exclusion of the PEG blocks can be attributed to the unfavorable low entropy of the mixing of rods and coils according to the Flory lattice theory [22,31]. This theory predicts that the ordered nematic structure formed by rod/coil/solvent mainly consists of the rods and rejects the coils with high selectivity. However, as the order of the rod packing decreases, the coils could be accepted in the nematic structure. In the present condition, the combination of the polypeptide block and PEG blocks in the same molecule prevents the macrophase separation, and microphase separated structure, i.e., spindle-like supramolecular structure is formed as shown in

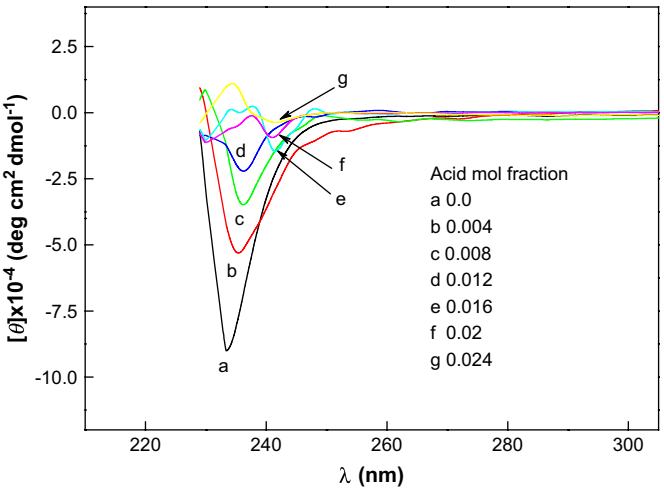
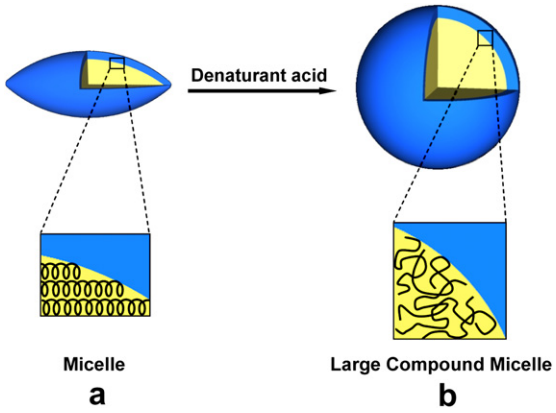


Fig. 4. CD spectra for the PBLG-*g*-PEG aggregate solutions with various TFA mole fractions.



Scheme 1. Schematic representation of the model proposed for the PBLG-*g*-PEG aggregates formed at different conditions.

Scheme 1(a). In such a structure, a bundle of helix rods aggregates in a nematic liquid crystal manner to form the core, while the PEG blocks protrude outside to form the shell. The dislocation between the parallel polypeptide blocks in the micelle core could result in a spindle-like shape.

When the denaturant acid is added, the random coil conformation is preferred. The polypeptide chain becomes flexible and tends to be randomly packed within the aggregate core, resulting in a decrease of the order of polypeptide block packing. The PEG chains become accepted in the less ordered structure. Due to both loose packing of the polypeptide chains and entrance of the PEG blocks to the core, the size of the aggregates turns larger. When the acid content is further increased, the helix completely transforms to the random coil. Consequently, large spherical micelle is formed as shown in Scheme 1(b). As revealed by our recent self-consistent field theory calculations [32], amphiphilic graft copolymer with hydrophobic backbone and hydrophilic grafts can self-assemble to form large compound micelle. The backbone and grafts are uniformly distributed in the core of the large compound micelle and surrounded by a shell outside of hydrophilic grafts. The large size micelle observed in the present experiments could be the large compound micelle as predicted by the theory. The random polypeptide chains and PEG chains are mixed to form the core, and some PEG blocks form the outside shell to stabilize the large compound micelles. Thus, in the above process, the supramolecular aggregate structure change is induced by the intramolecular helix–coil transition.

In summary, self-assembly behavior of PBLG-*g*-PEG in ethanol medium was studied by TEM, LLS, and CD. The experimental results revealed that the conformation change of the polypeptide graft copolymer exerts a marked effect on its self-association behavior. Spindle-like micelles with polypeptide chains aligned inside the cores were formed in ethanol solution without denaturant acid. When the denaturant acid is added, the rigid α-helix transforms to random coil and the coiled polypeptide chains and PEG chains may pack randomly within the micelle cores, surrounded by the PEG chains outside. As a result, large compound micelles with spherical morphology are formed. The helix–coil conformation transition of synthetic polypeptide is an interesting and important research topic. The understanding of the influence of such intramolecular conformation change on its supramolecular assembly could be helpful for knowing the self-association behavior of more complex polypeptide systems.

Acknowledgments

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