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Morphological Variation of Peptide-synthetic Hybrid Block Copolymer Assemblies in Nonaqueous Media

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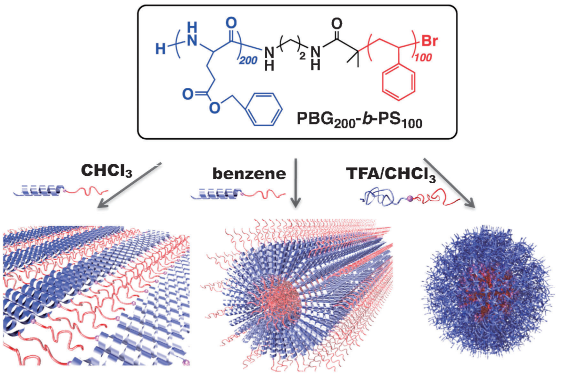
In this communication, we demonstrate that significantly different aggregation morphologies are readily generated from a simple poly(γ-benzyl L-glutamate)/polystyrene hybrid block copolymer in nonaqueous media. These nanostructural variations were governed basically by both the solubility of each block to solvents and the character of the polypeptide block such as helix rod–coil transition.

Block copolymers composed of structurally-regulated biopolymers and conventional synthetic polymers provide promising opportunities to design novel self-assembled materials with nanoscaled structures. For example, employment of artificial peptide as one of the block segments permits an easy design for rod–coil-shaped molecules. In the α-helix, especially, the peptide bond planes are parallel to the axis of the helix. In this geometry, each peptide bond forms intramolecular hydrogen bonds and the side chains are on the outside of the spiral structure. As a result, α-helical peptide behaves as a rigid and stiff rod-like molecule whose diameter and length can be precisely controlled by choosing the kind of amino acids and by changing the degree of polymerization, respectively. The self-organization of this type of block copolymer (rod–coil type) is governed not only by phase-separation as was observed in classical coil–coil type but also by the direct interaction among the rigid rod–segments, which allows production of supramolecular assemblies with characteristic nano- and microscaled morphologies.[1–5] A pioneer work on conjugation of synthetic polymers with polypeptides was reported by Gallot and coworkers,[1] who employed poly(γ-benzyl L-glutamate)(PBG) as a helical polypeptide and polybutadiene as a flexible synthetic polymer, and found the spontaneous formation of lamellar structure in solid state. Recently, several research groups[6–11] have also focused on the conjugation of synthetic polymers with peptides in order to fabricate more precisely controlled self-organization systems. Schlaad's group has used similar diblock polymers to Gallot's containing PBG block and polystyrene block in place of polybutadiene block, and showed the detailed aggregation morphology in solid state.[12] Klok and co-workers have prepared similar diblock oligomers containing PBG block and oligo(styrene) in place of polystyrene block, and revealed that the self-organization of these oligomers can be manipulated via changes in the block length ratio and also via temperature-induced conformational changes in the peptide segment.[13,14] In an aqueous system, the same research group has reported that the block copolymer of poly(L-glutamic acid)-*b*-polybutadiene forms vesicular assemblies and the size and shape of the assemblies can be manipulated by changing the pH value and ionic strength.[15] Other researchers have developed the stimuli-responsive morphological variation, so-called "schizophrenic" assemblies, of block copolymers in aqueous media.[16,17]



(a) *Lamella* (b) *Rod-like micelle* (c) *Spherical micelle*

Figure 1. Morphological variation on the PBG_{200}-*b*-PS_{100} self-organization readily accomplished by changing the solvents: $CHCl_3$ (a), benzene (b), and 13% TFA-containing $CHCl_3$ (c).

We report here on the self-organization of a poly(γ-benzyl L-glutamate)-*b*-polystyrene (PBG-*b*-PS) in nonaqueous media (Figure 1). Our aim is to exploit the nanostructural variation induced with the conformation of the peptide block and the difference in solubility of each block in organic solvents. Previously we demonstrated that the rod-like, helical PBG assembles spontaneously in organic solvents such as $CHCl_3$, in which PBG takes α-helical conformation, on the basis of antiparallel helix–helix interaction induced with helix macrodipole moment.[18,19] The helical polypeptide is well known to generate the macrodipole, that is, the electrostatic potential directed from the N-terminus to the C-terminus.[20,21] This helix-macrodipole effect would play an important role in fabricating an anisotropic nanostructure. In addition, if in nonaqueous media PBG-*b*-PS could form nanostructures having versatile morphologies depending on the conformation and solubility of each block, it would become much easier to fabricate a well-defined, topologically-controlled solid surface at nanolebel and also to apply it to nanodevices and surface-coating reagents that need a dry process.

The diblock copolymers were prepared via two steps by using a bifunctional initiator that can initiate polymerization of both vinyl monomers through ATRP and of α-amino acid *N*-carboxyanhydrides (NCA) through ring-opening polymerization (also see Supporting Information[2]). Styrene was first polymerized by ATRP using this initiator, $Cu^{I}Br$, and tetramethylcyclam. After removal of the protective group (Boc) from the resulting polystyrene chain end, the polymerization of γ-benzyl L-glutamate NCA was performed to yield PBG-*b*-PS.

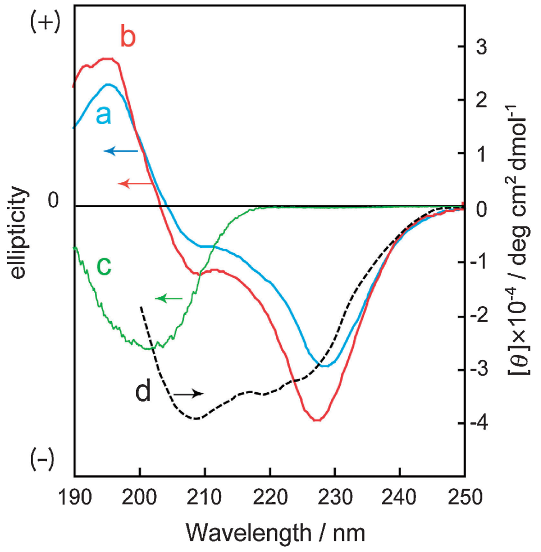


Figure 2. CD spectra of the cast films of PBG_{200}-*b*-PS_{100} prepared from $CHCl_3$ (a), benzene (b), and TFA(13 vol %)/$CHCl_3$ solutions (1 wt %) (c), and the HFIP solution of PBG_{200}-*b*-PS_{100} ([peptide unit] = 0.5 mM) (d).

The number average degree of polymerization of the PBG block was about 200 and about 100 for the PS block (PBG_{200}-*b*-PS_{100}). The α-helix content of this block copolymer in hexafluoroisopropyl alcohol (HFIP) was evaluated to be 99% by circular dichroism spectroscopic (CD) analysis.[23–27]

In order to examine the solubility of each polymer block in organic solvents, the corresponding homopolymers (PBG and PS) were prepared elsewhere, whose degree of polymerizations were almost comparable to those of blocks in the copolymer. We employed three solvents ($CHCl_3$, benzene, and trifluoroacetic acid (TFA)) by considering the solubility of each block and the secondary structure of PBG block. $CHCl_3$ behaved as good-solvent for both homopolymers and induced α-helical conformation of the PBG block. Benzene was nonsolvent for PBG but good-solvent for PS, and TFA was reversely good-solvent for PBG but nonsolvent for PS.

The secondary structures of the PBG_{200}-*b*-PS_{100} cast films prepared from $CHCl_3$ (Figure 2a) and benzene solutions (1 wt %) (Figure 2b) were investigated by means of CD measurements. Both spectra are similar and show a typical aggregated α-helix pattern, as evidenced by the red-shifting of the 222 nm band toward around 230 nm and the flattening of the 208 nm band,[23] compared with the spectrum obtained in HFIP where the block copolymer must take α-helical conformation in monomeric state (Figure 2d). TFA is good-solvent for PBG but is known to behave as an α-helix breaking solvent.[28] In fact, when ^{1}H NMR spectra of PBG_{200}-*b*-PS_{100} were measured in the mixed solvents of $CDCl_3$ and TFA, the peak at 3.9 ppm due to α-helix observed in $CDCl_3$ was found to shift to a lower magnetic field of 4.6 ppm by TFA addition, and the further addition up to 13 vol % of TFA converted α-helix to random coil conformation completely (Figure S3[22]). Its cast film also shows a typical CD pattern of randon coil conformation (Figure 2c).

The supramolecular organization of the PBG_{200}-*b*-PS_{100} block copolymer in various organic solvents was subsequently investigated by means of AFM and TEM observations. Figures 3Aa and 3Ab show a tapping-mode AFM phase image obtained by casting from $CHCl_3$ solution (1 wt %), in which the PBG block was, as mentioned above, in an aggregated α-helix conformation. The structure is obviously lamellar and is characterized by parallel strips alternatively bright and dark (Figure 3Ab). The AFM phase images can be used to distinguish between rigid and soft surfaces based on the difference in micromechanical properties such as aggregation force and viscoelesticity of the sample surface. It is thus considered that the bright strips contain the rod-like, helical PBG blocks and the dark strips are formed by PS coils. The cross-sectional analysis also supported such an assignment: the width of a bright and of a dark strip were determined to be about 30 and 28 nm, respectively, and these values are well consistent with those of the helix length of PBG block (approximately 30 nm) and the average extended length of PS coil (approximately 33 nm). In addition to these AFM analyses, further consideration of the helix-macrodipole effect, namely, the head-to-tail antiparallel orientation of the PBG helices, which is energetically more favorable, suggests that a lamella morphology is most likely as illustrated in Figure 1a.

The cast film from the benzene solution of PBG_{200}-*b*-PS_{100} contained an aggregated α-helix structure (Figure 2a) and thus the self-assembling properties in this solvent are also expected to be governed with the rod-like helical components. An AFM height image is displayed in Figure 3Ba. The sample was prepared from benzene solution by casting onto mica substrate. Well-developed, worm-like aggregates are clearly observed, and their size and shape are relatively homogeneous. The length, width, and height of the aggregates are estimated to reach up to 500–1000, 100–150, and about 10 nm, respectively. The TEM micrograph obtained without staining (Figure 3Bb) shows tube-like morphology comprising the dark wall (about 30 nm thickness) and the bright inner (about 60 nm diameter), and this is supported by the above AFM analysis. Together with considerations of the molecular length of PBG_{200}-*b*-PS_{100} and its helical character, it can be concluded that a rod-like double-layer micelle model (Figure 1b) on the basis of the aggregated α-helix structure is more reasonable than a tube-like model, because the inner core should be occupied with PS coils. The solubility of each block to benzene also supports this model. It should be noted that observed height of the aggregate by AFM analysis was quite small (ca. 10 nm) compared to the width (100–150 nm). Probably the rod-like micelle structure collapsed vertically during the dry process for the cast film preparation.

Figure 3C shows the TEM analyses for the cast films prepared from the TFA(13 vol %)–$CHCl_3$ mixed solvent containing PBG_{200}-*b*-PS_{100} (1 wt %). From the micrograph in Figure 3Ca that was taken without staining, homogeneously dispersed circular aggregates with diameters of 200–350 nm are observed. The AFM image for the same sample (data not shown) revealed such aggregates to be a spherical. These results suggest the formation of a core–shell type micelle structure, in which the PBG coils are located on the shell and the core is loaded with PS coils, by considering the solubility of each block to TFA. To prove this structure more directly, TEM observation was performed with Cu^{2+}-staining[22] (Figure 3Cb) since Cu^{2+} is predicted to bind specifically peptide bonds. Ring-shaped assemblies appeared, whose black ring and inner white part

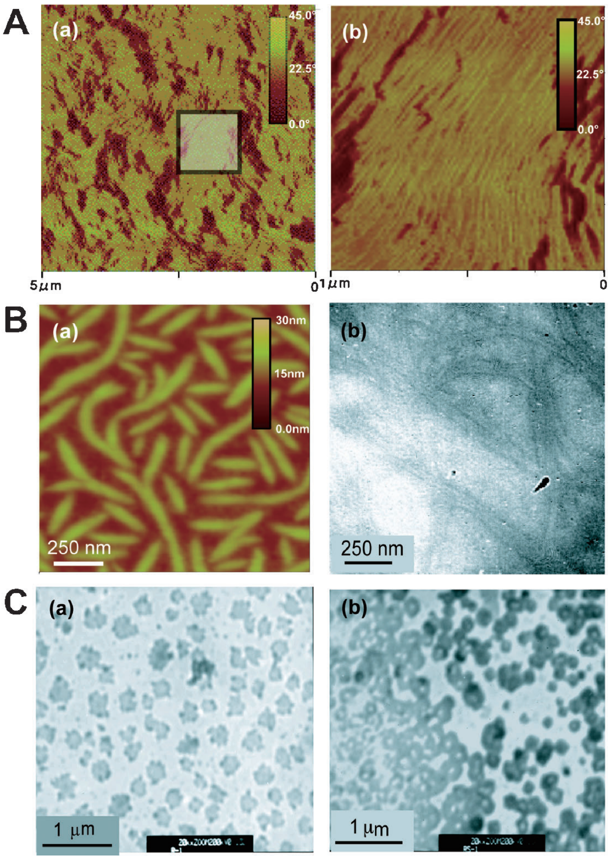


Figure 3. Self-organization of PBG$_{200}$-b-PS$_{100}$ in various solvents. (A) AFM image (phase) obtained from $CHCl_3$ solution (a) and that at higher resolution of the area highlighted in the picture of the left (b). (B) AFM image (height) (a) and TEM picture obtained by casting from the benzene solution without staining (b). (C) TEM images for the cast films obtained from the 13% TFA-containing $CHCl_3$ solution without staining (a) and with Cu^{2+}-staining (b).

can be surely assigned to the Cu^{2+}-bound PBG shell and the PS-loaded core, respectively. In addition, the diameter of the Cu^{2+}-stained micelles (250–350 nm) is consistent with that of Cu^{2+}-free ones.

Furthermore, these nanostructured surfaces gave a variety of surface wettability depending on their characteristic topologies. The film surfaces of PBG$_{200}$-b-PS$_{100}$ obtained both from $CHCl_3$ and TFA(13 vol %)–$CHCl_3$ solutions afforded relatively hydrophobic surfaces with water contact angles of $75 \pm 3°$. On the other hand, a drastic increase in hydrophobicity was observed for the cast film from benzene ($100 \pm 2°$).

In summary, we have demonstrated that different aggregation morphologies are obtainable from a simple homopolypeptide–PS hybrid block copolymer. The PBG$_{200}$-b-PS$_{100}$ diblock copolymer formed well-defined lamella morphologies based on a specific inter-helix interaction in the cast film from $CHCl_3$. Addition of TFA caused a drastic transformation from lamella to core–shell micelle structures. Dissolution into benzene led to such a unique morphology as rod-like double-layer micelles depending on both the specific inter-helix interaction and the solubility of each blocks. It should be noted that the solid surfaces covered with versatile nanostrucutres can be readily generated from our peptide–polymer hybrid simply by changing the solvents.

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