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Gelation of Helical Polypeptide–Random Coil Diblock Copolymers by a Nanoribbon Mechanism**

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The polypeptide poly(γ-benzyl-L-glutamate) (PBLG) serves as a model rod-like polymer in solution and in the solid state because of its rigid α-helix conformation.[1,2] The stiff rod-like conformation of the PBLG helix is a crucial prerequisite for its unique solution behavior such as liquid crystalline ordering[3,4] and thermoreversible gelation.[5] Nematic ordering (orientational order of rod-like molecules) is responsible for the thermoreversible gelation of PBLG in dilute solution. Diblock copolymers of PBLG and flexible organic polymers are unable to form a nematic phase due to the presence of a block with a bulky random-coil conformation. Here we report the discovery of thermoreversible gelation of diblock copolymers of PBLG in dilute solution which has not been observed previously despite many studies of such materials.[6] We also explain the gel formation of PBLG-based diblock copolymers in solution by a mechanism which is distinct from the current understanding of PBLG self-assembly.[7] Based on our findings, we describe the formation of self-assembled functional scaffolds with precisely aligned fluorescent labels. The results illustrate a novel and general mode of self-assembly for block copolymers of PBLG and presumably other helical polypeptides and demonstrate an attractive new approach to nanostructured supramolecular materials derived from biological and synthetic polymers.

Our initial studies involved the synthesis of poly(ferrocenylsilane)-*b*-poly(γ-benzyl-L-glutamate) (PFS-*b*-PBLG), conjugates of an organometallic polymer and polypeptides with narrow molecular weight distributions (Figure 1a, Table 1).

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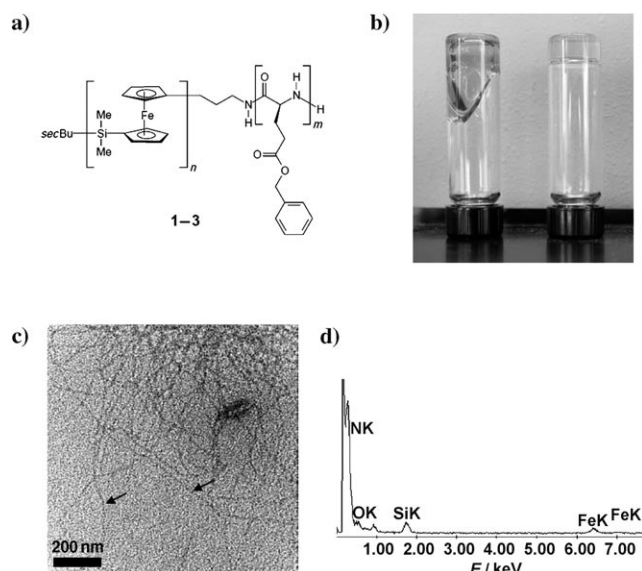
Figure 1. a) Chemical structure of PFS-*b*-PBLG. Subscripts *n* and *m* denote the average number of repeating units in the polymer backbones. b) Toluene solution of **1** (3 wt%) below the critical gelation concentration (3.3 wt%) (left) and toluene gel of **2** at 0.2 wt% (right). c) TEM image of the toluene gel of **2** (1 wt%). Arrows in the picture indicate the individual fibers with about 20 nm width. d) EDX spectrum of the unstained toluene gel of **2**.

Table 1: Characterization and gelation characteristics of block copolymers.

Block copolymers[a]	M_n[b] [g mol^{-1}]	M_w/M_n[c]	C_{gel}[d] [wt%]	T_{gel}[e] [°C]	L_{helix}[f] [nm]	W_{ribbon}[g] [nm]
PFS$_{38}$-PBLG$_{69}$ (**1**)	24 500	1.14	3.3	48	10.3	14.9
PFS$_{38}$-PBLG$_{95}$ (**2**)	30 200	1.13	0.2	50	14.2	15.8
PFS$_{19}$-PBLG$_{48}$ (**3**)	15 300	1.21	1.9	46	7.2	9.7
PS$_{93}$-PBLG$_{76}$ (**4**)	26 700	1.14	0.3	48	11.4	19.0
PEG$_{227}$-PBLG$_{56}$ (**5**)	22 300	1.18	0.5	45	8.4	16.0

[a] Subscripts indicate the average number of repeating units of the polymer determined by ^{1}H NMR integration. PFS: poly(ferrocenyldimethylsilane), PS: polystyrene, PEG: poly(ethylene glycol), PBLG: poly(γ-benzyl-L-glutamate). [b] Molecular weights of the block copolymers measured by ^{1}H NMR integration. [c] Polydispersity indices of molecular weights of block copolymers obtained by GPC with THF/[Bu$_4$N]Br (0.003 M) as the eluent. [d] The apparent critical gelation concentrations for the toluene solutions of the block copolymers. [e] The gel–solution transition temperature measured with the toluene gels at C_{gel}. [f] Calculated length of the PBLG helix of the block copolymers. L_{helix} (in nm) = $N_{PBLG} \times 0.15$ nm where N_{PBLG} is the average number of residues in the PBLG helix determined by ^{1}H NMR. [g] The width of the ribbon in the dried gels measured by SAXS.

These materials were synthesized by ring-opening polymerization (ROP) of γ-benzyl-L-glutamate-*N*-carboxyanhydride (γ-Bz-L-Glu-α-NCA) with amine-terminated PFS (amino-PFS) as a macroinitiator.[8] Amino-PFSs were prepared by living anionic ROP of a strained [1]-silaferrocenophane monomer.[9,10] The PFS-*b*-PBLG block copolymers were very soluble in common organic solvents such as chloroform and tetrahydrofuran (THF), but only sparingly soluble in hot *N,N*-dimethylformamide (DMF).

We found that PFS-*b*-PBLG block copolymers are soluble in hot toluene and, to our surprise, form optically transparent gels upon cooling to ambient temperature. The gelation was thermally reversible in all cases. The apparent critical concentration for the gelation (C_{gel}), below which the solution exists as a viscous fluid (Figure 1b), strongly depends on the chain length of the PBLG block (see Experimental Section for details of the measurement). For example, block copolymers **1** and **2**, which differ only in the number of γ-Bz-L-Glu residues (69 for **1** and 98 for **2**) in the PBLG block, have a considerably different C_{gel} (3.3 wt% for **1** and 0.2 wt% for **2**). This result indicates that the interaction between the PBLG helices is a crucial factor in the gelation process. The lower C_{gel} of block copolymer **3** as compared to that of **1** (Table 1) suggests that the chain length ratio between the PFS and the PBLG blocks might also be a factor in the gelation process. In addition, for PFS$_{38}$-*b*-PBLG$_{133}$ (38.5 kDa), where the chain length ratio between the PFS and the PBLG exceeds 1:3, incomplete gelation was observed (macroscopic phase separation into a dense block copolymer gel and the free solution phase) regardless of the concentration of the block copolymer in toluene. Significantly, the gels retained their shape and thermoreversibility on the addition of methanol (5 vol% to toluene). This suggests that there is no end-to-end intermolecular hydrogen bonding between the PBLG blocks, as methanol can readily disrupt the hydrogen bonding between the PBLG chains.[11,12] This result indicates that hydrogen bonding is not a key factor for the gelation.

PBLG homopolypeptide is known to form thermoreversible gels in various solvents such as toluene and benzyl alcohol,[13] which has led to challenging questions on the origin of this phenomenon.[14] PBLG differs from other gel-forming polymers[15] because its rigid-rod conformation does not allow the formation of chain–chain entanglements. One aspect of the current understanding of the gelation of PBLG in solution is based on phase separation.[16] The PBLG homopolypeptide undergoes microscopic phase separation when a warm homogeneous solution in the dilute concentration regime (< 2 wt%) is cooled. Phase separation increases the concentration of PBLG locally, and the increased concentration forces the rods to order in a liquid crystalline phase where PBLG rods align parallel to the long axis of the phase-separated domains. Strong dipolar π–π interactions between the phenyl groups of the PBLG rods consequently stabilize the microfibers that are intertwined into a 3-D network structure.

Based on the mechanism described above, it is difficult to imagine that block copolymers of PBLG and a random-coil polymer could form a gel since the block copolymers can not be ordered in a liquid crystalline phase in dilute solution due to the presence of the bulky random-coil polymer chain at the end of PBLG helix. Our findings reported here imply that a different mechanism of gelation is required to explain the self-assembly behavior of PBLG–random coil diblock copolymers in solution. Therefore, we studied the detailed structure of the gels of PFS-*b*-PBLG in toluene with transmission electron microscopy (TEM), atomic force microscopy (AFM), and small-angle X-ray scattering (SAXS). TEM images of the dried gel of **2** formed in toluene (Figure 1c)

show a network of fibrous structures, where each individual fiber has a width of about 20 nm. Energy- dispersive X-ray spectroscopy (EDX) clearly evidenced the presence of both the PBLG block and PFS block in the structure (Figure 1 d). AFM experiments were carried out to determine the lateral dimension and the height profiles of the fiber (Figure 2 a and b). Analysis of the AFM height profiles (Figure 2 b) revealed
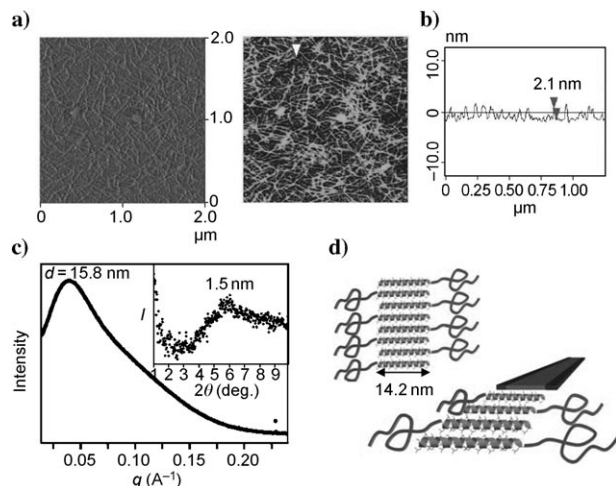
Figure 2. a) AFM phase (left) and height (right) images of the toluene gel of **2** (0.1 wt%). b) AFM height profile of the nanoribbons. c) SAXS profile of the dried gel of **2** showing the width of the ribbon (15.8 nm). The inset denotes the diffraction at 1.5 nm indicating the distance between two PBLG helices obtained by PXRD. d) A schematic presentation of the nanoribbon formed in the network structure of the toluene gel of **2**. 14.2 nm indicates the calculated length of the PBLG helix.

a height of 2.1 ± 0.2 nm, and a lateral dimension of about 23 nm. This suggests that the individual fibrous structure is not cylindrical but has a ribbon or tape-like morphology. SAXS measurements were carried out on the dried gel because all gels showed strong birefringence when examined by polarized optical microscopy, indicating the presence of regular structures (Figure 2 c). The results corroborated TEM and AFM observations of the width of the individual fibers by showing a diffraction peak at 15.8 nm. This lateral dimension of the fiber obtained on SAXS has not been observed in the gels of the PBLG homopolypeptide due to the lack of order in the diameters of fibers.[17] Powder X-ray diffraction (PXRD) of the dried gels of PFS-*b*-PBLG also showed a diffraction peak at 1.5 nm, which indicates the distance between PBLG helices.[4]

Based on the TEM, AFM, SAXS, and PXRD results, a mechanism for the gelation of **2** was conceived. From the experimental data and the molecular dimensions of block copolymer **2**, containing a PBLG helix of 14.2 nm length and 1.5 nm diameter, we propose that the observed fibers are nanoribbons assembled from the 1-dimensional stacking of the block copolymers in a monolayer fashion (Figure 2 d).[18] This self-assembly mechanism is reminiscent of that proposed for nanoribbons derived from dendron rod-coil molecules[19] but with two differences. First, in our case, the self-organizing

motifs are high molecular weight block copolymers, and second, hydrogen bonding interactions, which have been shown to be essential for dendron rod-coil self-assembly, are not involved. We propose that strong dipolar π–π interactions involving phenyl groups between the PBLG helices stabilize the structure and could be the driving force for the self-assembly.[20] The long axis of the PBLG helix is parallel to the plane of the ribbon. The PFS block protrudes outside of the ribbon due to its high solubility in toluene and prevents the aggregation between nanoribbons in the solution. In the nanoribbon, the PBLG chains are most probably oriented in an anti-parallel fashion. In this orientation the dipole moments of the PBLG helices are oriented in a favorable manner, and the steric repulsion between the PFS chains is minimized.[21] The nanoribbon mechanism proposed here explains the dependence of C_{gel} of the block copolymers on the length of the PBLG helix and the independence of gelation on the presence of hydrogen bonding disrupting solvents such as methanol.

The proposed mechanism is potentially applicable to other diblock copolymers when the appropriate structural requirements are met: a stable α-helical conformation of the PBLG polypeptide block and a random-coil block which is soluble in the given solvent. To establish the generality of the mechanism, we prepared two block copolymers, polystyrene-*b*-PBLG (PS-*b*-PBLG) **4** and poly(ethylene glycol)-*b*-PBLG (PEG-*b*-PBLG) **5**. We chose PS and PEG because these polymers possess a random-coil conformation in solution and are readily soluble in toluene. In addition, amino-functionalized PS and PEG are commercially available, and the block copolymers, PS-*b*-PBLG and PEG-*b*-PBLG, have been previously synthesized.[22,23] Both diblock copolymers formed stable transparent thermoreversible gels in toluene at concentrations as low as 0.3 wt% (Figure 3 a and c). A detailed structural characterization of gels in toluene of both block copolymers also showed structures of the fibers identical to those described for the PFS-*b*-PBLG gels. TEM observations and SAXS experiments revealed that both block copolymers self-assemble in a manner that is consistent with the nanoribbon mechanism described above (Figure 3 b and d). In addition to our work, diblock copolypeptides containing both random-coil and α-helical peptide blocks have been shown by Deming and co-workers to form hydrogels in aqueous solution.[24] Our results together with those of Deming strongly suggest that the gelation of the diblock copolymers with an α-helix and a random-coil block in an appropriate solvent (toluene for the PBLG block copolymers) is a general phenomenon, and the concept could be expanded to numerous block copolymers containing a helical polypeptide block other than the PBLG.

The ROP of α-NCA yields an amino end group at the polypeptide chain. This amino group could be further functionalized by reacting guest molecules such as fluorescent dyes, nanoparticles, and other functional species. The resulting self-assembled structures of the labeled block copolymers would then be functional scaffolds with precise alignment of the labels in the nanostructures. To test this idea, we labeled PEG-*b*-PBLG with a pyrene (Py) chromophore using an *N*-hydroxysuccinimide activated pyrene derivative (Figure 4 a
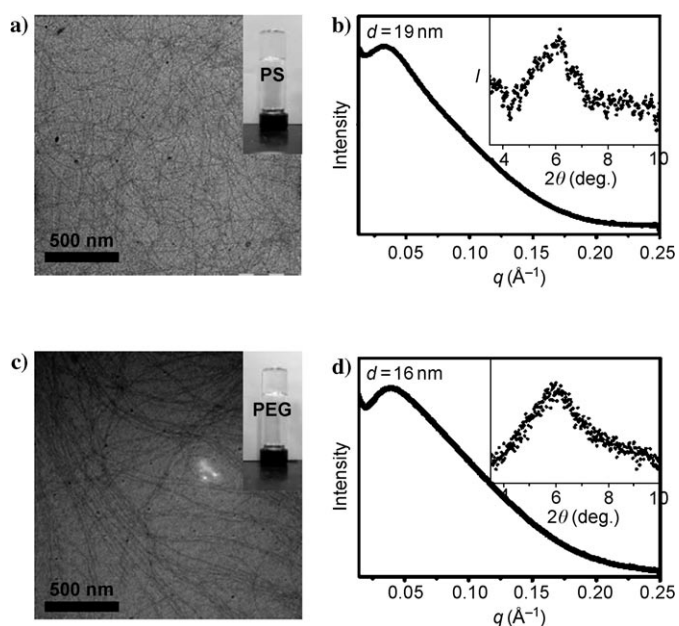
Figure 3. a,c) Energy-filtered TEM (EF-TEM) images of 1 wt% toluene gels of PS-*b*-PBLG **4** (a) and PEG-*b*-PBLG **5** (c). Insets show the transparent gels of the respective block copolymers formed at the critical gelation concentrations. b,d) SAXS profiles of dried gel for PS-PBLG **4** (b) and PEG-PBLG **5** (d). Insets are PXRD results of the dried gels indicating the distance between PBLG helices (1.5 nm) in the nanoribbon structure.
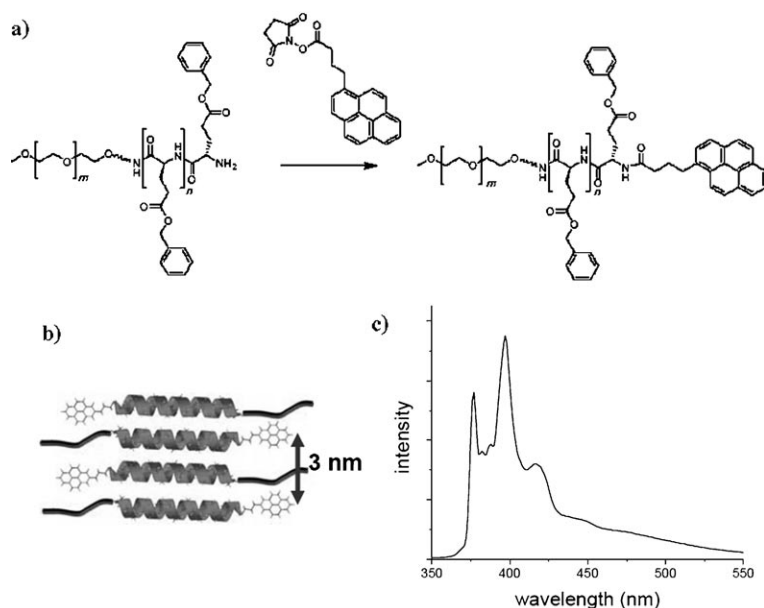
Figure 4. a) N-terminal labeling of PEG-*b*-PBLG. b) Graphic representation of the self-assembled nanoribbon of pyrene-labeled PEG-*b*-PBLG. c) Fluorescence emission spectrum (excitation at 340 nm) of toluene gel of pyrene-labeled PEG-*b*-PBLG.

and Experimental Section).[25] The functionalized PEG-*b*-PBLG formed a transparent thermoreversible organogel at 2.5 wt% in toluene. This indicates that the incorporation of bulky guest molecules such as pyrene does not hamper the self-assembling ability of the block copolymer and the self-

assembled structure can accommodate numerous functional molecules and nanoparticles with 1-D alignment at the defined location. For Py, the fluorescence emission spectrum reveals information about its micro-environment. Based on the mechanism proposed here, Py groups would be segregated in the nanoribbon structure by a 3 nm distance and shielded from one another by flexible PEG chain. Despite the high local concentration of Py groups, the structural model predicts that excimer formation should be strongly inhibited (Figure 4b). The excimer sandwich requires close proximity of two Py molecules (ca. 4 Å), achieved either through pre-association or diffusive motion of Py groups in the medium.[26] The fluorescence emission spectrum of the organogels of pyrene-labeled PEG-*b*-PBLG shows only characteristic monomeric emission peaks of pyrene (377 nm, 397 nm, and 416 nm) and no noticeable excimer emission at about 480 nm. This result provides strong support for the proposed mechanism. In addition, the unique self-assembly mechanism allows labeling species to be incorporated in the block copolymer without interfering with the self-assembly process. The labeled organogels based on random coil–helical polypeptide block copolymers could be utilized as scaffolds for catalysis.

The studies described here show that synthetic polymers with a defined conformation can play a role as well-defined building blocks for self-assembly. It is remarkable that the random coil–helical polypeptide diblock copolymers behave with the same precision as well-defined small molecules that form organogels (organogelators)[27] although the size of the block copolymer building blocks is orders of magnitude larger. This dimension regime is comparable to that of proteins that form elaborate 1-D structures such as F-actin.[28] Our findings could facilitate the development of synthetic polymeric systems that self-assemble precisely into well-defined nanostructures by adopting stable polymer conformations. Furthermore, fine-tuning of the properties and chemical functionality of organogels made of helix–random coil diblock copolymers should be possible based on the mechanism proposed here. The potential development of PFS-*b*-PBLG gels as magnetic and redox-active scaffolds where the additional functionality arises from the presence of metal atoms is particularly attractive.[29, 30]

Experimental Section

Synthesis of the block copolymers, gel characterization, and labeling experiments: Amino-PFSs and block copolymers of PBLG were synthesized as previously reported.[8] Amino-polystyrene ($M_n = 10500$ g mol^{-1}, PDI = 1.05) and amino-poly(ethylene glycol) ($M_n = 10000$ g mol^{-1}, PDI = 1.08) were purchased from Polymer Source. For organogels, block copolymers and toluene were mixed in a sealed vial and heated until the mixture became a homogeneous solution. The homogeneous solution was stored at 22 °C for 24 h. After 24 h, the organogel was subjected to gravity for 24 h. When no flow was observed, we defined the state as a gel. The lowest concentration of gelation in this condition was determined as C_{gel}. T_{gel}

was measured by the procedure reported by Hirst et al.[31] All C_{gel} and T_{gel} values were measured three times. Pyrene labeling of the block copolymer was achieved by means of an amidation reaction between PEG-*b*-PBLG and *N*-hydroxysuccinidal pyrene butyric acid (fivefold excess to the block copolymer) in THF at room temperature for 24 h. The completion of the reaction was monitored by ^{1}H NMR integration. Labeled block copolymer was purified by repeated precipitations in diethyl ether and washings over acetone until the washings show no presence of pyrene by UV/Vis absorption. Organogels of the labeled block copolymer were prepared in sealed quartz cells. Fluorescence emission spectrum of the organogel were taken on a Flurolog 322 fluorescence spectrophotometer with a 340 nm excitation wavelength.

Microscopy and X-ray experiments: Transmission electron microscopy (TEM) images were obtained on a Philips CM200 microscope operated at an acceleration voltage of 80 kV. The TEM specimen was prepared by gently placing a carbon-coated copper grid on the fraction of organogel. The TEM grid was removed after 30 sec and air-dried for 1 h. When necessary, the sample specimen was shadowed with Au/Pd (7–8 Å thickness at 20° tilting angle). Zero-loss bright field images were obtained on the energy-filtering TEM microscope Carl Zeiss Leo 912 Omega at an acceleration voltage of 120 kV. Small angle X-ray scattering (SAXS) experiments were carried out at the 4C1 X-ray beamline of Pohang Accelerator Laboratory (Pohang, Korea). The scattered data were collected using a 2-D CCD detector and the X-ray wavelength was 1.608 Å ($Co_{K\alpha}$ radiation). Powder X-ray diffraction data were collected on a Rigaku RINT-2000 counter diffractometer with a $Cu_{K\alpha}$ radiation source (operated at 40 kV, 40 mA). For X-ray experiments, organogels were slowly dried under air for 24 h. Atomic force microscopy was performed on a NanoScope III in the tapping mode. The sample preparation for AFM measurement was identical to the preparation of the TEM specimen. Fresh cleaved mica was used as a substrate. The height and width values of the fiber were determined from ten different locations.

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