Self-assembly of dendron-helical polypeptide copolymers: organogels and lyotropic liquid crystals†

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New macromolecular self-assembling building blocks, dendron-helical polypeptide copolymers, have been synthesized; these materials possess a well-defined 3-D shape and self-assemble in solution to form nanoribbon and lyotropic liquid crystalline phases.

The secondary structure of peptide chains plays a crucial role in the formation of the well-defined tertiary structure of proteins. Biological functions such as enzymatic activity arise from these specific tertiary structures. Unlike proteins, most synthetic polymers lack the ability to retain specific 3-D structures in solution or in the bulk phase due to their random coil conformations. Such irregular conformations hamper the potential of synthetic polymers as useful building blocks for the creation of self-assembled structures with well-defined size and 3-D shape. Therefore, rod–coil block copolymers which contain a well-defined rigid segment as a rod block in their architecture have received considerable attention due to their unique self-assembly behavior which differs from that of conventional coil–coil block copolymers.[1] Also, dendrimers and dendrons[2] have been adopted for the generation of novel polymeric architectures such as linear-dendritic block copolymers[3] because of their monodisperse and well-defined globular structures. Stupp and coworkers have reported dendron-rod coil (DRC) molecules and their self-assembly in solution.[4] The DRC molecules form nanoribbon structures similar to the self-assembly of organogelator molecules[5] in solution, in which directional non-covalent interactions such as hydrogen bonding provide a main driving force. These studies demonstrated the development of structurally well-defined self-assembling building blocks for which the self-assembly process could be highly directed and controlled.[6]

Poly(γ-benzyl-L-glutamate) (PBLG) forms a stable α-helix under various conditions.[7] This rigid α-helical conformation is responsible for the interesting phase behavior found for PBLG such as lyotropic liquid crystalline ordering.[8] Rod–coil block copolymers based on a rigid PBLG helix have been studied extensively for several decades.[9] Recently, we found that random coil-PBLG block copolymers with flexible polyferrocenylsilane blocks[10] form thermoreversible gels in toluene.[11] The underlying mechanism of the gelation of PBLG diblock copolymers was suggested to be different from that of the gelation of the PBLG homopolypeptide because the random coil-PBLG diblock copolymers could not form a liquid crystalline phase in dilute solution, which is essential for homo PBLG gelation.[12] Our proposed mechanism for the self-assembly of the random coil-PBLG block copolymers implies that a wide variety of PBLG block copolymers with novel architectures other than random coil-helix could generate well-defined supramolecular structures in solution. It appeared logical that two well-defined macromolecular architectures, dendritic and helical polymers, could be combined to generate polymeric building blocks with a well-defined 3-D shape, which dictates the self-assembly process and resulting supramolecular structures. Here we report the synthesis of dendron-helical polypeptide (DHP) copolymers and their self-assembly in solution.[13] The DHP copolymers form organogels as well as lyotropic liquid crystalline phases *via* the architecture-directed self-assembly.

The dendrons **1** and **2** were prepared from the alkoxy benzyl ether dendrons by treatment with tri(ethylene glycol) and ethylene diamine *via* 1,1′-carbonyldiimidazole (CDI)-activated coupling reactions.[14,15] The DHP copolymers were synthesized by the ring-opening polymerization (ROP) of γ-benzyl-L-glutamate *N*-carboxylanhydride (γ-Bn-Glu α-NCA) with the dendrons as macroinitiators. The polymerizations were performed in a THF/DMF mixture at room temperature for 72 h. The precipitation of the reaction solutions into diethyl ether gave the DHP copolymers in good yields. The DHP copolymers were characterized by ^{1}H NMR and gel permeation chromatography (GPC). The average number of amino acid units in the PBLG block was calculated by ^{1}H NMR integration (Table 1). The GPC results of the DHP copolymers gave exaggerated molecular weight values compared to the absolute molecular weights obtained from ^{1}H NMR integration. The rigid conformation of the PBLG helix is presumably responsible for the increased molecular weights

Table 1 Characterization and self-assembly characteristics of the dendron-helical polypeptide copolymers

	M_n[a]	PDI[b]	$DP_{n,PBLG}$[a]	C_{gel}[c] (wt%)	T_{gel}[c] (°C)	W_r[d] (nm)
3	8,220	1.18	30	2.9	41	5.5
4	13,480	1.18	43	1.8	49	7.1
5	21,300	1.21	78	0.3	52	11.7[e]

[a] Molecular weight and degree of polymerization calculated by ^{1}H NMR integration. [b] Molecular weight polydispersity index (M_w/M_n) measured by THF/[Bu_4N]Br (0.003 M) GPC. [c] Critical gelation concentrations in wt% and gel–solution transition temperature. [d] The width of the ribbon formed in toluene measured by SAXS. [e] The width of the PBLG helix calculated by ^{1}H NMR integration. (L_{helix} (nm) = $N_{PBLG} \cdot 0.15$ nm where N_{PBLG} is the average number of residues in the PBLG helix determined by ^{1}H NMR).

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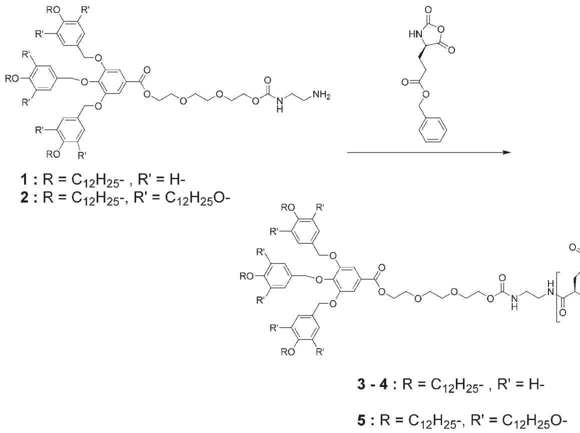
estimated by GPC. We confirmed the absence of aggregation of the DHP block copolymers in the eluent for the GPC experiments by dynamic light scattering.[10,15]



1 : R = $C_{12}H_{25}$- , R' = H-
2 : R = $C_{12}H_{25}$-, R' = $C_{12}H_{25}O$-

3 - 4 : R = $C_{12}H_{25}$- , R' = H-

5 : R = $C_{12}H_{25}$-, R' = $C_{12}H_{25}O$-

We observed thermoreversible gelation of the DHP copolymers in toluene. The DHP copolymer **4** having a PBLG helix with 43 amino acid residues formed a transparent gel at 1.8 wt% in toluene. The length of the PBLG helix has an important effect on the gelation of the DHP copolymers because the π–π interaction between the PBLG helices[11] is the main proposed driving force of the self-assembly and accounts for the stability of the assembled structures. We infer that hydrogen bonding between the DHP copolymers is not involved in the gelation because the gel retains the integrity and thermoreversibility in the presence of methanol, which disrupts hydrogen bonding.

The self-assembled structures formed by **3** and **4** in toluene were studied by transmission electron microscopy (TEM), atomic force microscopy (AFM), and small-angle X-ray scattering (SAXS) (Fig. 1). In both the AFM and TEM images, organogels of **3** and **4** showed bundles of fibers in which the unit fibers had a width of *ca.* 10 nm. In AFM height profiles,[15] these unit fibers had a height of *ca.* 2 nm, which indicates that the morphology of the fibrous structure is a flat ribbon formed by stacking of the DHP copolymers containing a PBLG helix (*ca.* 1.5 nm diameter) in a 1-D monolayer. From SAXS measurements on the dried toluene gels, we inferred that the width of the ribbon is 5.5 nm for **3** and 7.1 nm for **4**. This observation fits the molecular dimension of the DHP copolymers (the calculated lengths of the PBLG blocks are 4.5 nm for **3** and 6.4 nm for **4**). The SAXS of the gels also showed a peak corresponding to a spacing of 1.5 nm, indicating the distance between PBLG chains (Fig. 1d).

The experimental results strongly suggest that the nanoribbon mechanism for the self-assembly of random coil-helical polypeptide block copolymers[11] also applies to the DHP copolymers. We imagine that the DHP copolymers self-assemble in an anti-parallel fashion in order to minimize the steric hindrance between dendritic blocks and unfavorable orientation of the dipole moment of the PBLG helix[16] in the nanoribbon structures. The long axis of the PBLG helix is parallel to the plane of the ribbon. The anti-parallel stacking of the copolymers also could be inferred from the observation that the organogel from the DHP copolymer **5** with a bulkier dendritic block than other DHP copolymers also showed the same structural features in both TEM and AFM (Fig. 2). In an anti-parallel stacking mode, the distance between two neighboring dendritic blocks facing the same side is *ca.* 3 nm, which could accommodate the bulky dendrons in the planar nanoribbon structures without changing the morphology. This stacking of the DHP copolymers in a 1-D ribbon gives a *ca.* 2 nm height profile in AFM experiments. Due to the compact but bulky geometry in which all the peripheral groups are emanating from the core of the dendron, the dendritic block could prevent the DHP copolymers from aligning into a nematic phase during self-assembly in dilute solution, which was found in the homo PBLG gelation.

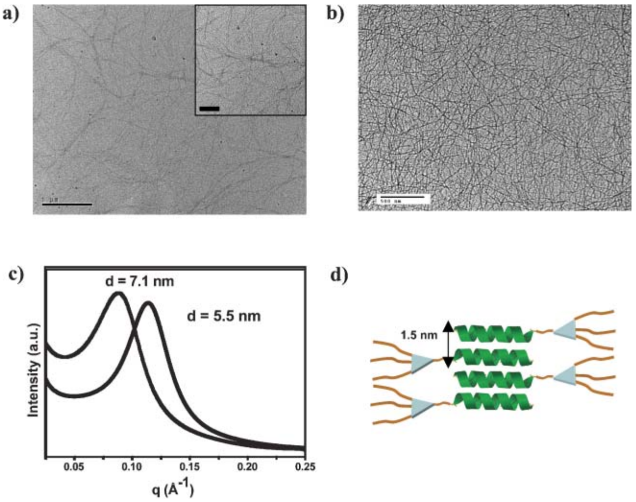


Fig. 1 a) TEM images of dried toluene gels of **3** (3.5 wt%). The scale bar represents 1 μm. The scale bar in the inset indicates 200 nm. b) TEM images of dried toluene gel of **4** (3 wt%). The scale bar indicates 500 nm. c) SAXS profiles for dried gels of **3** (d = 5.5 nm) and **4** (d = 7.1 nm). d) Schematic representation of the nanoribbon formed from the self-assembly of DHP copolymers. The arrow indicates the distance between two peptide helices in the nanoribbon structures.

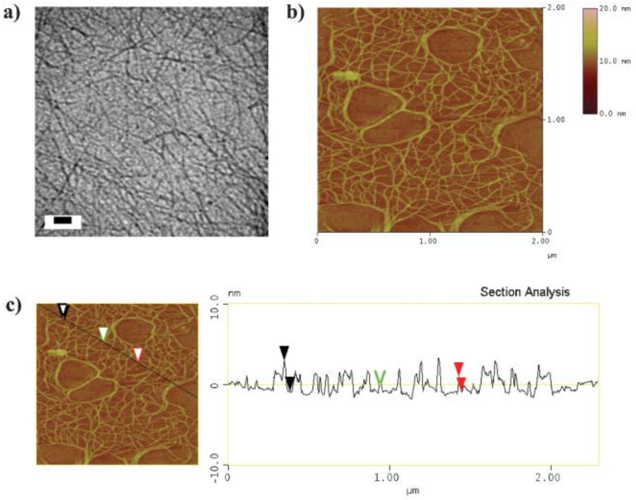


Fig. 2 a) TEM image of dried toluene gel of **5** (1 wt%). The scale bar represents 100 nm. b) AFM height images of dried toluene gel of **5** on a mica substrate. c) Sectional analysis of the AFM height image. Red arrows show 1.9 nm height for the individual ribbon. Green arrows show the width of the ribbon (15 nm) at the half height. Black arrows indicate the overlap of two ribbons and show a height of 3.9 nm.

For PBLG itself, a smectic liquid crystalline phase in solution is rarely observed, due to the polydisperse nature of PBLG made by conventional ROP of α-NCA. Tirrell and coworkers showed that

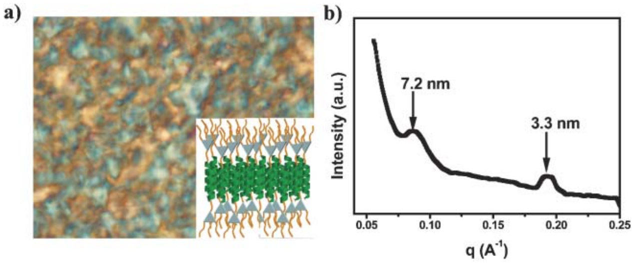


Fig. 3 a) Optical birefringent texture of THF solution (40 wt%) of **4** and proposed layered structures of liquid crystalline state of **4** (inset). b) SAXS profile of dried solid of THF solution (40 wt%) of **4**.

monodisperse PBLG prepared by a genetic engineering method undergoes smectic ordering in concentrated solution.[17] Gallot and coworkers demonstrated that PBLG block copolymers form lamellar structures in concentrated solution, in spite of the PBLG polydispersity, *via* hexagonal packing of the PBLG helices.[18] Lecommandoux *et al.* have reported thermotropic liquid crystalline states, including a smectic phase for dendron-rod molecules, which are based on the mesogenic rod molecules of the trimer and tetramer of biphenylester moieties.[19]

We anticipated that the DHP copolymers could form lyotropic liquid crystalline phases in concentrated solution because of the unique structural characteristics of the dendron block, which is flexible enough to stabilize the liquid crystalline phase of copolymer molecules and, at the same time, should not prevent the alignment of the helices into the liquid crystalline phase due to its compact chemical structure. Also, the DHP copolymers are different from conventional PBLG block copolymers because of the presence of the dendritic block which possesses a well-defined structure with no polydispersity in molecular weight.

When we examined concentrated solutions ($>$ 40 wt%) of the DHP copolymers **3** and **4** in THF and $CHCl_3$, we found these solutions showed a similar birefringent texture under the polarized optical microscope (Fig. 3). SAXS experiments on the dried solid from the concentrated THF solution of **4** showed two diffraction peaks at 7.2 nm and 3.3 nm, which suggests that **4** exhibits smectic-like order in concentrated solution.[20] We propose a layered structure of **4** (Fig. 3a inset) in the liquid crystalline phase that results from an anti-parallel stacking of **4** into a 2-D layer in a manner similar to the nanoribbon formation. This result strongly suggests that smectic ordering of the DHP copolymers in solution is feasible with a dendritic molecule as a non-random coil block even though the PBLG block is polydisperse in chain length.

The DHP copolymers reported here are distinguished from previously reported dendron-rod–coil (DRC)[4,6] and dendron-rod molecules[19] because of the macromolecular nature of the PBLG block compared to the mesogenic rigid rod molecules in DRC and dendron-rod molecules. The size of the PBLG helix, which could be up to *ca.* 15 nm (100 amino acid units) in length and 1.5 nm in diameter, is distinguished from the mesogenic oligo-biphenylester groups in the dendron-rod molecules. The polymeric nature of the PBLG helix and its defined conformation enable the block copolymer to self-assemble into well-defined supramolecular structures although the block copolymers only apparently use a weak non-directional interaction, π–π interaction, as a driving force of the self-assembly. It should be noted that thermoreversible gelation of the DHP copolymers implies the generality of the nanoribbon mechanism for the self-assembly of block copolymers of the helical polypeptides.[11] The architectural feature of the DHP copolymers also enables the smectic ordering of the copolymers in solution although the polydisperse nature of the DHP copolymers closely resembles homo PBLG. Also, the synthetic methodology used in this work could be readily applied to prepare various block copolymers with structural diversity in both the dendritic and polypeptide blocks.

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