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Poly(diacetylene)-nanofibers can be fabricated through photo-irradiation using natural polysaccharide schizophyllan as a one-dimensional mold

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Schizophyllan interacts with various 1,4-diphenylbutadiyne derivatives to induce their chirally-twisted packing. A series of referential experiments using other polysaccharides (amylose, pullulan, dextran, *etc.*) and a carbohydrate-appended detergent (dodecyl-β-D-glucopyranoside) indicates that these 1,4-diphenylbutadiyne derivatives are accommodated within a tubular cavity constructed by a helical superstructure of schizophyllan. In these 1,4-diphenylbutadiyne derivatives, 1,4-bis(*p*-propionamidophenyl)butadiyne can be easily polymerized through UV-irradiation, in which schizophyllan acts as a one-dimensional mold to produce the corresponding poly(diacetylene)s with fibrous morphologies. Detailed investigations on this unique approach to prepare the nanofibers revealed that it includes two individual processes, that is, 1) UV-mediated polymerization of encapsulated 1,4-bis(*p*-propionamidophenyl)butadiyne to produce immature nanofibers and 2) their reorganization through hydrophobic interfiber interactions into ordered nanofibers. The other 1,4-diphenylbutadiyne derivatives could not be polymerized through UV-irradiation, indicating that the *p*-propionamido-functionalities play substantial roles for a suitable packing of the monomer for the polymerization. The other 1,4-diphenylbutadiyne derivatives, however, can be also polymerized through γ-ray irradiation in the presence of schizophyllan to give the corresponding poly(diacetylene)-nanofibers, emphasizing the wide applicability of the schizophyllan-based strategy for polymerization of various 1,4-diphenylbutadiyne derivatives.

Introduction

Convenient approaches to design conductive nanofibers have received an increasing research interest, since such nanofibers can be used as conductive wires in nano-scaled electric circuits in a coming age. There are two attractive candidates for such nanofibers. One is a family of covalently-bonded π-conjugated polymers including poly(acetylene)s,[1] poly(phenylenethynylene)s,[2] *etc.* and the other is a family of noncovalently-assembled nanofibers of low molecular-weight organic compounds having donor–acceptor complexes (*e.g.*, tetracyanobenzoquinone (TCNQ) and tetrathiafulvalene (TTF))[3]. The π-conjugated polymers have one clear advantage over the latter: that is, the stable conductivity is generated even under harsh conditions, *e.g.*, at high temperature where the molecular assemblies are easily decomposed. However, they also have a drawback as well: that is, simple polymerization of the corresponding monomers usually results in amorphous polymer-aggregates, in which the polymer strands are highly entangled in a random fashion. It is strongly desired, therefore, to establish convenient strategies to fabricate the nanofibers, in which individual polymers are aligned in a parallel orientation. Such nanofibers are expected to be useful molecular wires having excellent conductivity through the long axis.

Poly(diacetylene)s are a family of the most interesting research targets among the π-conjugated polymers, since they are readily produced through photo-irradiation (UV or γ-ray) without any initiators.[4] It is known, however, that closely-packed pre-organization of the corresponding monomers is indispensable for their photo-mediated polymerization (topochemical polymerization). Poly(diacetylene)s can be, therefore, usually prepared from molecular assemblies (crystals, micelles, Langmuir–Blodgett films, *etc.*) of the corresponding monomers, in which the monomers are aligned in a parallel but slightly slided packing mode suitable for the topochemical polymerization. Since the resultant morphologies of obtained poly(diacetylene)s strikingly depend on the superstructure of the monomer-assemblies before photo-irradiation, pre-organization of the corresponding monomers into well-designed fibrous architectures is, therefore, a prerequisite to obtain the nanofibers composed of poly(diacetylene)s. Several research groups have devoted their intense research efforts on the fabrication of such fibrous monomer-assemblies, mainly utilizing the amphiphilic diacetylene-monomers which are spontaneously assembled into the fibrous superstructures.[5]

Schizophyllan (SPG, Fig. 1-a) produced by fungus *Schizophyllum commune* is an extracellular β-1,3-glucan having a β-1,6-glucoside-appendage at every three repeating units. This native polysaccharide has been of great interest for many researchers because of its anticancer activity as well as its gel-forming ability.[6] The most interesting structural feature of SPG is a reversible and solvent-induced structural transition between a triple-stranded helical structure (t-SPG) in water and individual single-strands (s-SPG) in dimethyl sulfoxide (DMSO).[7]

In a series of intense research on SPG, we found that SPG has a one-dimensional (1D) cavity inside its helical superstructure (Fig. 1-b) and accommodates various hydrophobic guests within this 1D cavity through the structural transition from s-SPG to

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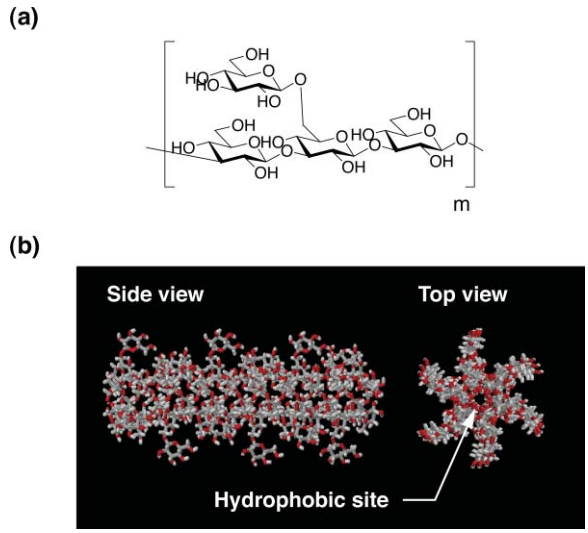


Fig. 1 (a) Chemical and (b) spatial structure of schizophyllan having a triple-stranded helical structure (t-SPG).

t-SPG (renaturation process).[8] It should be noted that this renaturation process is essential for the guest encapsulation: in fact, no complexation can be observed by simple mixing of t-SPG with the guests. We assumed that the 1D cavity inside the rigid-helical structure of t-SPG is not ready for the complexation. However, once SPG takes the random-coiled structure (s-SPG), the 1D cavity is exposed to the solvent and the complexation can occur during the water-induced renaturation process. One may regard, therefore, that SPG can be induced to fit the guests of various size and shape through this unique renaturation process and in this respect, the complexation behaviour of SPG is quite different from that of cyclodextrins, which have strict size- and shape-selectivity.

The first example of SPG–guest complexes was observed for single-walled carbon nanotubes (SWNTs).[9] In this example, highly-stable and water-soluble SPG–SWNT complexes are formed by mixing s-SPG in DMSO with a SWNT-dispersed aqueous solution. Atomic force microscopic (AFM) observations of the resultant complexes revealed that the SWNTs are accommodated within the 1D cavity of SPG. We also reported that not only SWNTs but also polyaniline (PANI) can form a stable macromolecular complex with SPG through the renaturation process.[10] Of great interest, the resultant SPG–PANI complex has a fibrous superstructure, in which several PANIs are packed in a parallel fashion within the helical superstructure of SPG. This unique morphology is quite in contrast to free PANI, which gives only amorphous aggregates. This finding clearly indicates that SPG can act as a 1D host to accommodate not only rod-like polymers (SWNTs) but also flexible synthetic polymers (PANI) within the 1D cavity to produce the fibrous polymeric assemblies. More recently, we also found that small Au-nanoparticles can be entrapped by SPG to produce the 1D Au nano-arrays.[11] These findings consistently support the view that SPG can act as a general 1D host to accommodate a wide variety of guest metals and molecules to produce their 1D architectures.

Our research efforts are now focused on low molecular-weight compounds as guests.[12] Especially, it is of great significance to establish SPG-templated polymerization of various low molecular-weight monomers in the 1D cavity to construct the corresponding polymers with fibrous morphologies.[13] Herein, we report one of such successful examples that SPG can accommodate various 1,4-diphenylbutadiyne derivatives (DPBs) within the 1D cavity and can produce poly(DPBs)s-assembled nanofibers through UV or γ-ray irradiations (Fig. 2).

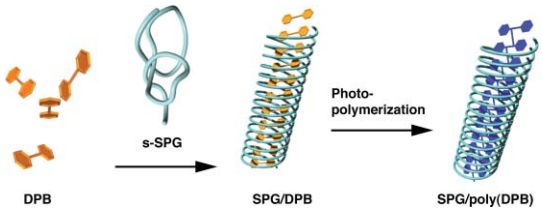
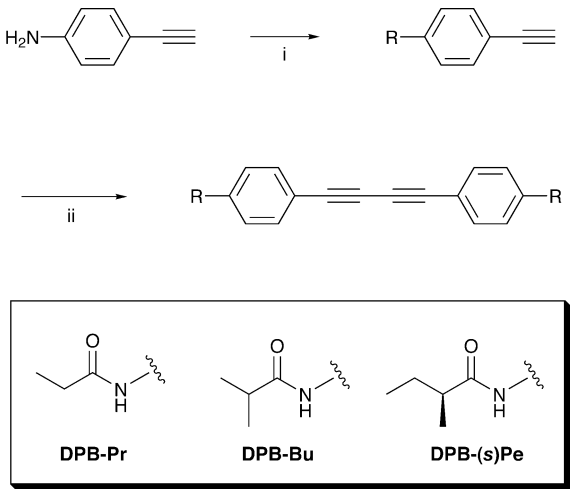


Fig. 2 Schematic illustration of our concept to use SPG as a 1D host to accommodate DPBs within the helical superstructure of SPG and construct poly(DPBs)-nanofibers through UV/γ-ray irradiation.

Results and discussion

Synthesis of DPBs

DPBs were prepared through 1) amido-coupling of a commercially available 4-ethynylaniline with the corresponding acid chlorides or acid anhydrides, followed by 2) Cu(II)-catalyzed homo-coupling of the resultant 1-amido-4-ethynylbenzene derivatives (Scheme 1). We prepared 1,4-bis(*p*-propionamidophenyl)butadiyne (DPB-Pr), 1,4-bis(*p*-*sec*-butyramidophenyl)-butadiyne (DPB-Bu) and 1,4-bis(*p*-(*S*)-2-methylbutyramidophenyl)butadiyne (DPB-(*S*)Pe) through this simple 2-step synthesis. Structural proofs of the resultant DPBs were obtained from ^{1}H NMR, MALDI-TOF-MS and IR spectral evidence. Along with these DPBs, we also used commercially available 1,4-diphenylbutadiyne (DPB) having no *p*-substituent in our experiments.



Scheme 1 Synthesis of 1,4-diphenylbutadiynes having propionamido (DPB-Pr), *sec*-butyramido (DPB-Bu), and (*S*)-2-methylbutyramido-substitutions (DPB-(*S*)Pe): i) propionyl chloride for DPB-Pr, isobutyric anhydride for DPB-*i*Bu, or (*S*)-(+)-methylbutyric anhydride for DPB-(*S*)Pe, pyridine, rt, ii) Cu(acetate)$_2$, pyridine, reflux.

Preparation of SPG–DPB complexes

SPG ($M_w = 150$ kDa) and DPBs were dissolved into DMSO and the resultant DMSO solution containing s-SPG (random-coiled single-strand) and DPBs was mixed with water to regenerate the t-SPG helical structure (the final water content was 70 v/v%). Formation of the desired SPG–DPBs complexes was confirmed by their circular dichroism (CD) spectra. For example, SPG–DPB-Pr complex gave a CD spectrum showing a negative Cotton signal (Fig. 3-a, bold green line), clearly indicating that SPG interacts with DPB-Pr monomers to arrange them in a chirally-twisted packing. It should be noted that the resultant aqueous DMSO solutions containing SPG–DPB complexes are slightly turbid, but the linear dichroism is negligibly small.

We carried out a few reference experiments using different solvent systems and polysaccharides in order to clarify the detailed mechanism of the interaction. The CD spectra of the

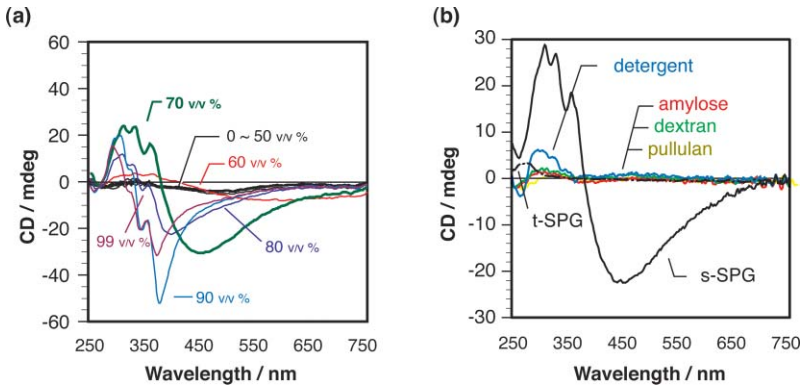


Fig. 3 (a) CD spectra of DPB-Pr in the presence of s-SPG under various aqueous DMSO solutions with different water contents and (b) CD spectra of DPB-Pr in the presence of s-SPG, amylose, dextran, pullulan, t-SPG and the detergent: $d = 1.0$ cm, 20 °C, $[H_2O] = 70$ v/v%, [DPB-Pr] = 25 μg ml^{-1}, [polysaccharide] or [detergent] = 25 μg ml^{-1}, 24 h after sample preparation.

SPG–DPB-Pr complex was dramatically changed by a change in the water content (Fig. 3-a). This solvent-dependent CD spectral change is considerably complex, however, two apparent characteristics can be seen. 1) The CD signal was observed only under water-rich conditions ($[H_2O] = 60\sim99$ v/v%), suggesting that the hydrophobic interactions, rather than the hydrogen-bonding interactions, play major roles for the complexation. This finding supports our assumption that hydrophobic DPBs are entrapped within the 1D cavity of SPG, as already reported for other guest molecules (SWNTs, PANI, Au-particles, *etc.*). 2) All CD spectra observed herein show negative Cotton-effects, indicating that DPB-Pr monomers are aligned in a left-handed helical manner within the 1D cavity.

Reference experiments using other polysaccharides (amylose, pullulan and dextran) also offered useful information on the mechanism of the interaction between SPG and DPB-Pr (Fig. 3-b). No other polysaccharide can induce any CD spectral change of DPB-Pr, indicating that a few factors (hydrophobic interactions, conformational changes, *etc.*), other than the hydrogen-bonding interactions, play substantial roles in the arrangement of DPB-Pr monomers in the 1D cavity of SPG.

Comparison between s-SPG and t-SPG also gave important information on the mechanism of the interaction: that is, when t-SPG (in water) was mixed with DPB-Pr (in DMSO), the resultant mixed solution gave no CD signal, indicating that the structural transition from s-SPG to t-SPG is essential for the interaction. This finding is of great significance because it implies that DPB-Pr molecules locate inside the helical structure of renascent t-SPG, as already reported for other guest molecules.

We also carried out an additional experiment using a carbohydrate-appended detergent (dodecyl-β-D-glucopyranoside) as a reference. DPB-Pr shows a small (compared with that in the presence of SPG) but perceptible induced-CD signal at around 280 nm in the presence of the detergent, indicating that DPB-Pr molecules are chirally packed within a hydrophobic inner space of the micelles having the chiral head groups. This finding also supports our assumption that the hydrophobic interactions play substantial roles for the complexations.

We also carried out CD spectral measurements on other DPBs including DPB, DPB-Bu and DPB-(*S*)Pe to find that these DPBs also showed SPG-induced CD signals, although their intensities are quite different (Fig. 4). These data indicate that their interactions with SPG are strikingly dependent on the structure of DPBs. For example, as shown in Fig. 4-a, the CD intensities of DPB-Bu and DPB are 3- and 10-fold weaker, respectively, than that of DPB-Pr. Comparison between DPB-Pr and DPB suggests an important role of *p*-amido-substituents for the interactions. We assume that the *p*-amido-substitutions are indispensable for arranging DPB-Pr molecules in the chirally-twisted packing, presumably through the hydrogen-bonding interaction with the neighbouring DPBs and/or SPG. Furthermore, comparison between DPB-Pr and DPB-Bu shows that bulky-substituents strongly disrupt the close and stable packing of DPBs in the 1D cavity.

UV-mediated polymerization of DPBs

UV-mediated polymerization of DPBs in the presence of SPG was carried out under two different conditions: that is, using 1) water-jacketted or 2) water-unjacketted cells. The temperature of the reaction cell was controlled at 25 °C by using the water-jacketted cell, however, it was elevated up to *ca.* 50 °C on UV-irradiation without using the water-jacketted cell. Before these experiments, we thought that the controlled temperature would play an important role to fabricate ordered nanofibers with uniform diameters, however, we found that the UV-mediated

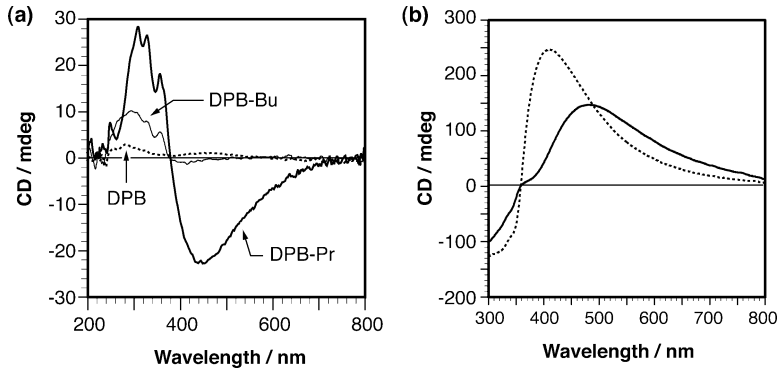


Fig. 4 (a) CD spectra of (dotted line) SPG–DPB complex, (plane line) SPG–DPB-Pr complex, and (thin line) SPG–DPB-Bu complex and (b) those of (plane line) SPG–DPB-(*S*)Pe complex and (dotted line) free DPB-(*S*)Pe: $d = 1.0$ cm, 20 °C, $[H_2O] = 70$ v/v%, [DPB], [DPB-Pr], [DPB-Bu], or [DPB-(*S*)Pe] = 25 μg ml^{-1}, [SPG] = 25 μg ml^{-1}, 24 h after sample preparation.

polymerization without using the water-jacketted cell could generate more ordered fibers than those produced under the controlled temperature (25 °C).

In the UV-mediated polymerization without using the water-jacketted cell, SPG–DPB-Pr complex in aqueous DMSO was placed at 5 cm distance from a high pressure Hg lamp and the temperature of the reaction solution was, therefore, elevated up to *ca.* 50 °C. A gradual colour change in the reaction solution could be visibly detected during the UV-irradiation process, as shown in Fig. 5-a. To our great interest, the solution colour changed from colourless to pale red during the first 4 h UV-irradiation and then gradually turned into pale blue during the following 16 h UV-irradiation. The time-course of the UV-vis spectra (Fig. 5-b) shows a biphasic spectral change during the UV-irradiation. In the first phase, an absorption band at 540 nm which is characteristic of poly(diacetylene)s appears. In the following phase, however, this new peak is gradually diminished and another peak appears simultaneously at 720 nm. Usually, such red-shifted absorption bands can be assigned to poly(diacetylene)s having the extremely large molecular weight and/or tight inter-stranded packing. We can exclude the former, because an isosbestic point can be observed in this spectral change. If the further UV-irradiation facilitates elongation of the corresponding poly(diacetylene) strands, the peak should continuously red-shift from 540 to 720 nm and the isosbestic point should not exist. We assumed, therefore, that this spectral change in the second phase arises from the inter-stranded packing of the poly(diacetylene) strands. The mechanism of this colour change will be further discussed below in detail.

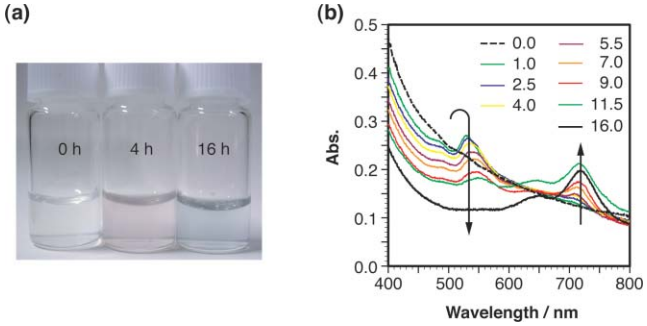


Fig. 5 Solutions of SPG–DPB-Pr complex after (left) 0, (centre) 4, and (right) 16 h UV-irradiation and (b) UV-vis spectra of SPG–DPB-Pr complex after 0, 1.0, 2.5, 4.0, 5.5, 7.0, 9.0, 11.5, and 16 h UV-irradiation: 25 °C, $d = 1.0$ cm, aqueous DMSO ($[H_2O] = 70$ v/v%).

The UV-mediated polymerization of DPB-Pr in the presence of SPG was also confirmed by the Raman spectra (Fig. 6), in which the SPG–DPB-Pr complex shows a sharp peak at 2000 cm^{-1} assignable to poly(diacetylene)s (–CH=CH-stretching vibration).[14] The difference between 4 and 16 h UV-irradiation does not induce any significant shift of the Raman peaks, again supporting that the elongated UV-irradiation time does not result in the further growth of the poly(diacetylene) strands. It should be emphasized that no such Raman peak appeared without SPG, indicating that SPG accommodates DPB-Pr within the 1D cavity to align them in a one-dimensional packing mode suitable for the topochemical polymerization.

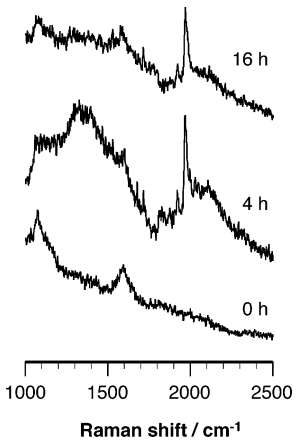


Fig. 6 Raman spectra of DPB-Pr in the presence of SPG after 0, 4 and 16 h UV-irradiation: cast films.

Other DPBs could not be polymerized through the UV-irradiation, although these DPBs are accommodated within the 1D cavity. These results closely correlate to the intensity of their SPG-induced CD signals: that is, the DPBs showing weak CD signals are not suitable monomers for the UV-mediated polymerization, presumably owing to their loose packing mode in the 1D cavity.

Transmission electron microscopic (TEM) observation showed that the resultant SPG–poly(DPB-Pr)s complex has a fibrous structure (Fig. 7-a) with diameters ranging from 2 to 20 nm. On the other hand, no such fibrous assembly was obtained without SPG (data not shown). We also confirmed that neither other polysaccharides (amylose, dextran, pullulan and t-SPG) nor the carbohydrate-based detergent (dodecyl-β-D-glucopyranoside) can produce such nanofibers (Fig. 7-c, d, e and f). These data clearly demonstrate an advantage of SPG as the 1D host to generate the nanofibers.

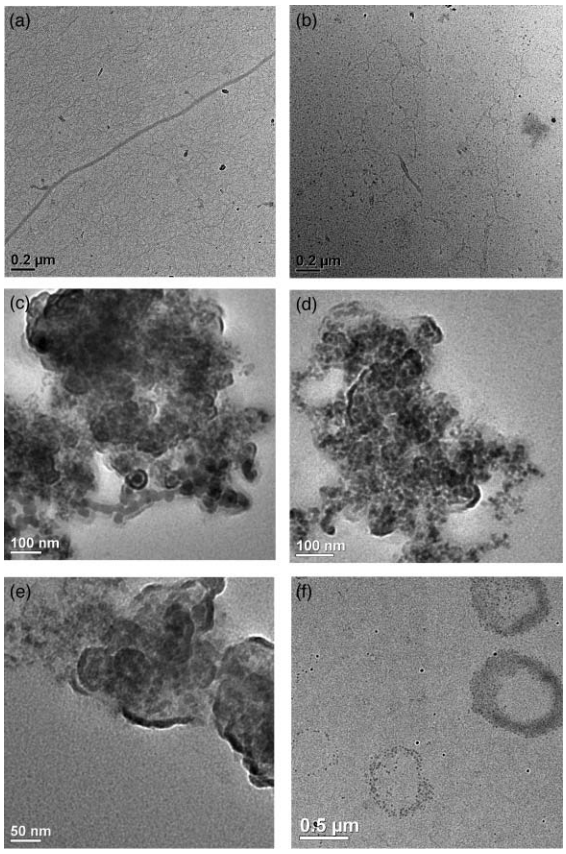


Fig. 7 TEM images of poly(DPB-Pr)s in the presence of (a) SPG (after 16 h UV-irradiation), (b) SPG (after 4 h UV-irradiation), (c) amylose, (d) dextran, (e) pullulan, and (f) dodecyl-β-D-glucopyranoside.

Energy dispersive X-ray (EDX) spectroscopic analysis showed the existence of oxygen and nitrogen in the nanofibers. Although the spectrum was overlaid by an enormous amount of carbon originating from the carbon grid, the analytical data gave an excess amount of oxygen in comparison to that of nitrogen. Since poly(DPB-Pr) itself contains an equal amount of oxygen and nitrogen, the presence of the excess amount of oxygen suggests that SPG strands co-exist around poly(DPB-Pr)-nanofibers. The co-existence of SPG strands is also supported by a morphological

change in the nanofibers through an acid-catalyzed degradation of the co-existing SPG. This result is discussed in the following section in detail.

Detailed investigations on the UV-mediated polymerization

Ordered nanofibers with uniform diameters can be observed for SPG–poly(DPB-Pr) complexes after 16 h UV-irradiation, however, shortened irradiation time (4 h) gives much different morphologies, that is, short and disordered nanofibers (Fig. 7-b). Together with the UV-vis spectral change during the polymerization suggesting the interfiber interactions, it is likely that the disordered nanofibers are organized into ordered ones during the UV-irradiation. We assumed that this structural transition should arise from temperature-induced re-organization of the poly(DPB-Pr) strands. As mentioned above, when the reaction cell was not water jacketted, the temperature of the reaction solutions was elevated up to *ca.* 50 °C on the UV-irradiation. This elevated temperature should disorder the helical superstructure of SPG around the as-grown poly(DPB-Pr) strands and then, the resultant poly(DPB-Pr) strands, which are partially exposed to the solvent, should be packed through interstrand hydrophobic interactions.

Comparison between the UV-mediated polymerization with or without the water-jacketted cell clearly supports this assumption: that is, when we used a water-jacketted reaction cell to keep the solutions at 25 °C, UV-irradiation gave a red-coloured solution and no further colour change from red to blue was observed even after 16 h UV-irradiation. The UV-vis spectra of the resultant solution confirm that no blue peak appears (Fig. 8, bold line). Furthermore, incubation of the resultant red-coloured solution in the dark at 50 °C resulted in an appearance of a new absorption peak at around 720 nm (Fig. 8, thin lines). These data also clearly support our assumption that the colour change from red to blue is induced through the temperature-induced re-organization of poly(DPB-Pr) strands.

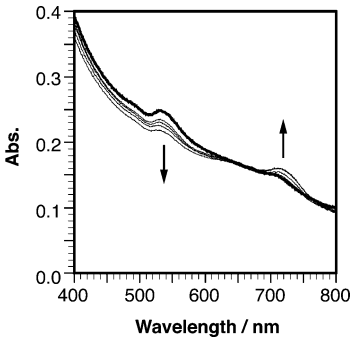


Fig. 8 Temperature-induced UV-vis spectral change (0 to 256 min) of SPG–poly(DPB-Pr) complexes in aqueous DMSO ([H_2O] = 70 v/v%): The original red-coloured solution was obtained through 16 h UV-irradiation on SPG–DPB-Pr complex using a water-jacketted (25 °C) reaction cell: 25 °C, $d = 1.0$ cm.

We also confirmed the effects of SPG on the ordered nanofibers by removing SPG from the SPG–poly(DPB-Pr) complexes through an acid-catalyzed degradation of SPG. As shown in Fig. 9-a, SPG–poly(DPB-Pr) complexes show a UV-vis spectrum having an intensified peak at 720 nm after the acid-catalyzed degradation. The removal of SPG from the complex, which results in poly(DPB-Pr) exposed to the media, should cause tight inter-stranded packings. TEM observation of the resultant complexes after the acid-catalyzed degradation did not show the ordered nanofibers but highly entangled net-like morphologies, supporting our assumption that perturbation (or removal) of the helical superstructure of SPG results in inter-stranded packing of poly(DPB-Pr) and the resultant colour and morphological changes.

γ-Ray mediated polymerizations of DPBs

As we mentioned above, the UV-mediated polymerization is only applicable for DPB-Pr, and no other DPBs, such as DPB, DPB-Bu and DPB-(*S*)Pe can be polymerized to afford the corresponding nanofibers. We, therefore, carried out γ-ray mediated polymerization of these DPBs, since γ-ray irradiation is known to produce poly(diacetylene)s very readily in comparison to the UV-irradiation. The aqueous DMSO solutions containing these SPG–DPBs complexes turned into pale blue after the γ-ray irradiation, indicating that the polymerization of all these DPBs does take place. Their UV-vis spectra after the γ-ray irradiation showed small new peaks around 500 nm (emphasized within closed circles, Fig. 10). Although these peaks are quite weak, they clearly indicate that these monomers can be polymerized through the γ-ray irradiation. TEM observation of the resultant SPG–poly(DPBs)s complexes showed fibrous superstructures that are similar to that of SPG–poly(DPB-Pr) complexes (Fig. 11-a). On the contrary, γ-ray-irradiation on DPBs in the absence of SPG gave amorphous aggregates with disordered rectangular shapes (Fig. 11-b). These rectangular poly(diacetylene)s should arise from amorphous aggregates of the corresponding "water-insoluble" monomers in the aqueous solution. These data clearly indicate that our SPG-based approach is a quite general one to obtain poly(diacetylene)-nanofibers from various DPB monomers.

Chirality of the nanofibers

It is of great interest to prepare poly(diacetylene)s with the helical superstructure, since they should be useful to develop various catalytic and sensory devices for chiral materials. In this respect, a conformation of poly(DPB-(*S*)Pe) is quite interesting. Poly(DPB-(*S*)Pe) prepared through the SPG-templated γ-ray irradiation followed by a dialysis with DMSO (to remove the unreacted monomer) showed a CD spectrum having a positive CD signal at around 300 nm that is assignable to π–π* transition of the phenyl-appendage (Fig. 12). On the other hand, no CD signals can be observed at 540 or 720 nm that are assignable

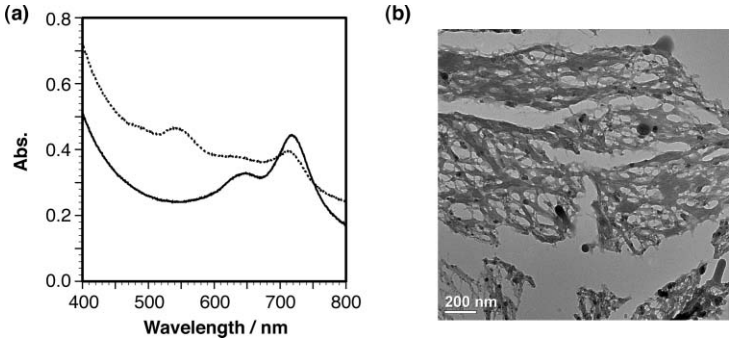


Fig. 9 (a) UV-vis spectra of SPG–poly(DPB-Pr) complexes (dotted line) before and (plane line) after the acid-catalyzed degradation of SPG and (b) TEM image of poly(DPB-Pr)s after the degradation followed by dialysis (MWCO 8000, water): for UV-vis, $d = 1.0$ cm, 25 °C.

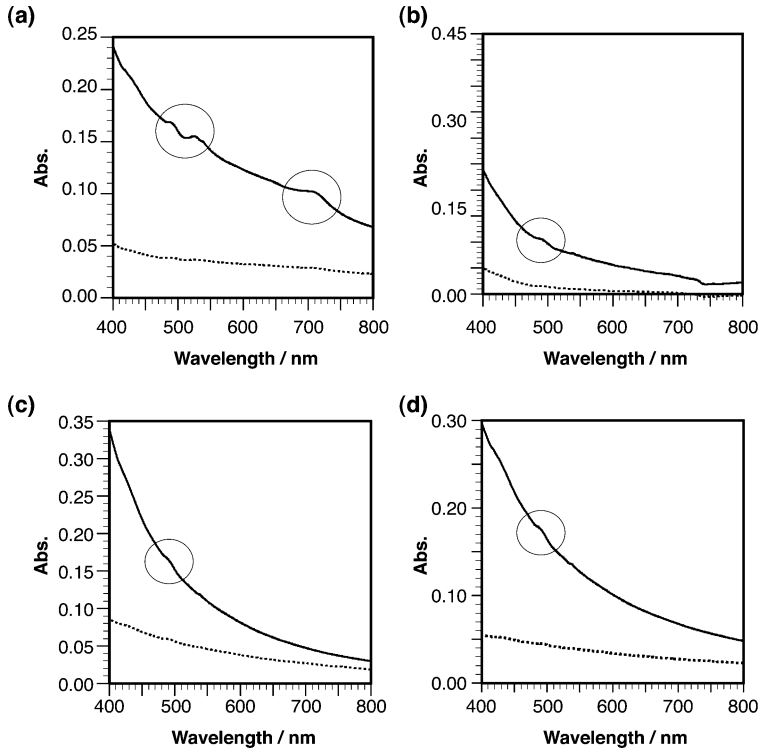


Fig. 10 UV-vis spectra of (a) DPB-Pr, (b) DPB-Et, (c) DPB-Bu and (d) DPB-(*S*)Pe after γ-ray irradiation in the (plane lines) presence and (dotted line) absence of SPG: 25 °C, $d = 1.0$ cm, aqueous DMSO ($[H_2O] = 70$ v/v%).

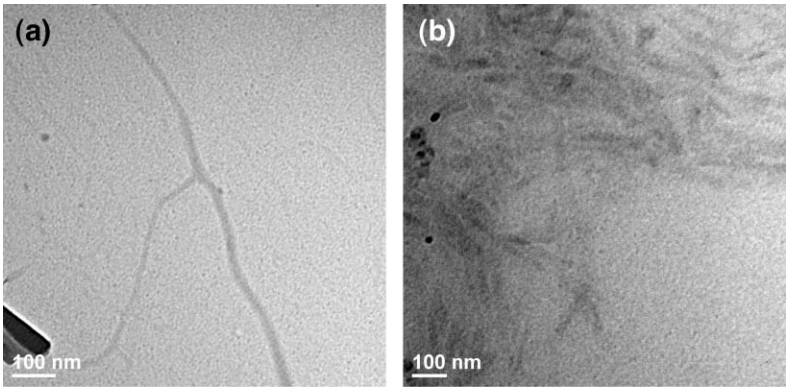


Fig. 11 TEM images of poly(DPB-(*S*)Pe)s in the (a) presence and (b) absence of SPG after γ-ray irradiation followed by dialysis (MWCO 8000, water).

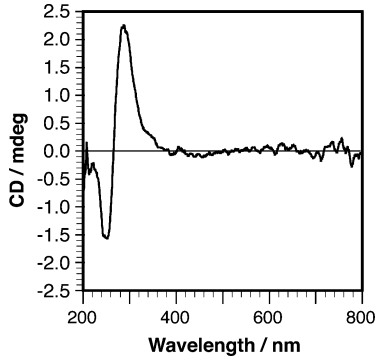


Fig. 12 CD spectra of poly(DPB-(*S*)Pe): $d = 1.0$ cm, 20 °C, in DMSO, the sample after the dialysis (DMSO) was directly used for this UV-vis measurement and therefore [poly(DPB-(*S*)Pe)] is unknown.

to π–π* transition of the polymer main chain. These data indicate that packing and conformation of the polymer main chain are not twisted in spite of the chiral substituents on the phenyl-appendages. We assume that tight inter-stranded packings between the polymer main chains would prohibit chiral twisting of the main chains.

Conclusions

We established a unique, easy and general approach to prepare nanofibers composed of various poly(DPBs)s. In our strategy, SPG acts as a unique 1D host to accommodate various DPBs to produce the corresponding poly(DPBs)s having highly ordered fibrous superstructure through UV- or γ-ray-irradiation. Recent studies on the fabrication of polydiacetylene-nanofibers utilize the corresponding monomers that are self-assembled into the fibrous superstructure. These diacetylene monomers should be carefully designed for their pre-organization into the nanofibers before polymerization. On the other hand, the SPG-templated approach can be applicable to various DPBs having various size and functionalities. Together with a series of our reports on SPG to show its unique properties as 1D hosts, the results we reported in this paper clearly show that SPG can be also applicable to template polymerization of the encapsulated monomers to produce the corresponding polymer-fibers. We believe that, along with the tolerance of SPG to the guests,

the SPG-templated approach should be a potent one to prepare nanofibers with other functional polymers.

Experimental

General

^{1}H NMR spectra were acquired on a Brucker DRX600 (Brucker Co., Ltd) in DMSO-d_6 at 600 MHz. The chemical shifts are reported in ppm (δ) relative to Me_4Si. IR spectra were recorded on a Perkin Elmer Spectrumone Fourier transform infrared spectrometer attached to a Universal ATR Sampling Accessory. Circular dichroism (CD) spectra were measured on JASCO 720WI Circular Dichroism Spectrometer. Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on PerSeptive Biosystems Voyager-DERP Biospectrometry Workstation. Silica gel 60 N (particle size 40–50 μm) for column chromatography was purchased from KANTO CHEMICAL Co. INC. Thin layer chromatography (TLC) was carried out with Merck TLC aluminium sheets pre-coated with silica gel 60 F_{254}. Native schizophyllan ($M_w = 1.5 \times 10^5$) was kindly supplied by Mitsui SeitoCo. Ltd., (Japan). The other chemicals were purchased from Aldrich.

Syntheses of 1-amido-4-ethynylbenzenes

1-Propionamido-4-ethynylbenzene. To 4-ethynylaniline (0.50 g, 4.27 mmol) and triethylamine (0.5 ml) in dichloromethane (30 ml) propionyl chloride (0.45 ml, 5.12 mmol) was added and then, the resultant mixture was stirred for 5 min. The resultant mixture was diluted with dichloromethane and the resultant organic layer was washed with water, dried over anhydrous magnesium sulfate, filtrated, and evaporated to give 1-propionamido-4-ethynylbenzene as a pale yellow powder (0.70 g, 94%). ^{1}H NMR (DMSO-d_6, TMS): 10.00 (s, 1H), 7.61 (d, $J = 8.40$ Hz, 2H), 7.40 (d, $J = 8.52$ Hz, 2H), 4.06 (s,1H), 2.35 (m, 2H), 1.08 (t, $J = 7.2$ Hz, 3H); IR (KBr, cm^{-1}) 3288, 2977, 2104, 1670, 1597, 1531; Anal. Calcd. for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 75.90; H, 5.90; N, 7.92%; $[M + Na]^+ = 196.06$ (calc. 196.07).

1-*sec*-Butyramido-4-ethynylbenzene. To 4-ethynylaniline (0.69 g, 5.89 mmol) in anhydrous pyridine (40 ml) isobutyric anhydride (30 ml) was added and then, the resultant mixture was stirred for 3 h. The resultant mixture was diluted with ethyl acetate and then, the organic layer was washed with water, 1 M HCl (aq.), and $NaHCO_3$ saturated aqueous solution, repeatedly. The resultant organic layer was evaporated and then, hexane was added to the resultant syrup. The resultant precipitate was washed with hexane several times to give the pure product in a yellow solid (0.75 g, 80%). ^{1} H NMR (DMSO-d_6, TMS): 9.99 (s, 1H), 7.74 (d, $J = 8.46$ Hz, 2H), 7.39 (d, $J = 8.46$ Hz, 2H), 4.27 (s, 1H), 2.59 (m, 1H), 1.09 (d, $J = 6.60$ Hz, 6H); IR (KBr, cm^{-1}) 3316, 2106, 1666, 1530; Anal. Calcd. for $C_{24}H_{24}N_2O_2$: C, 77.39; H, 6.49; N, 7.52. Found: C, 66.80; H, 6.47; N, 7.62%; $[M + H]^+ = 188.10$ (calc. 188.12).

1-(*S*)-2′-Methylbutyramido-4-ethynylbenzene. To 4-ethynylaniline (0.20 g, 1.7 mmol) and triethylamine (0.56 ml, 4.0 mmol) in pyridine (20 ml) (*S*)-(+)-2-methylbutyric anhydride (0.40 ml, 2.0 mmol) was added and then, the resultant mixture was stirred overnight. The resultant mixture was diluted with dichloromethane and the resultant organic layer was washed with water, 1.0 M HCl (aq.) and $NaHCO_3$ saturated aqueous solution and then, dried over anhydrous magnesium sulfate, filtrated, and evaporated to give 1-(*S*)-2′-methylbutyramido-4-ethynylbenzene as a pale yellow powder (0.12 g, 36%). ^{1}H NMR (DMSO-d_6, TMS): 10.00 (s, 1H), 7.63 (d, $J = 8.70$ Hz, 2H), 7.40 (d, $J = 8.64$ Hz, 2H), 4.06 (s, 1H), 2.40 (m, 1H), 1.60 (m, 1H), 1.39 (m, 1H), 1.07 (d, $J = 6.78$ Hz, 3H), 0.85 (t, $J = 7.34$ Hz, 3H); IR (KBr, cm^{-1}) 3292, 2963, 2105, 1771, 1506; Anal. Calcd. for $C_{24}H_{24}N_2O_2$: C, 77.97; H, 7.05; N, 6.99. Found: C, 77.41; H, 7.16; N, 6.92%; $[M + H]^+ = 202.12$ (calc. 202.11).

Syntheses of 1,4-bis(*p*-amidophenyl)butadiynes

1,4-Bis(*p*-propionamidophenyl)butadiyne (DPB-Pr). To 1-propionamido-4-ethynylbenzene (0.40 g, 2.31 mmol) in a mixture of anhydrous pyridine (30 ml) and methanol (50 ml) copper(II) acetate monohydrate (1.15 g, 5.47 mmol) was added and then, the resultant mixture was refluxed for 2 days under nitrogen atmosphere. The resultant reaction mixture was diluted with ethyl acetate and then, the organic layer was washed with NH_4Cl-saturated aqueous solution. The resultant organic layer was dried over anhydrous magnesium sulfate, filtrated, evaporated. The residue was purified on silica-gel (hexane : ethyl acetate = 5 : 1) to give 1,4-bis(*p*-propionamidophenyl)butadiyne (0.32 g, 80%) in a pale yellow powder. ^{1}H NMR (DMSO-d_6, TMS): 10.11 (s, 2H), 7.64 (d, $J = 8.70$ Hz, 4H), 7.50 (d, $J = 8.46$ Hz, 4H), 2.36 (m, 4H), 1.07 (s, 6H); IR (KBr, cm^{-1}) 3235, 3080, 2144, 1660, 1588, 1521; $[M + H]^+ = 344.12$ (calc. 344.15).

1,4-Bis(*p*-*sec*-butyramidophenyl)butadiyne (DPB-Bu). To 1-*sec*-butyramido-4-ethynylbenzene (0.50 g, 2.67 mmol) in anhydrous pyridine (50 ml) copper(II) acetate (0.97 g, 5.34 mmol) was added and then, the resultant mixture was stirred at 50 °C for 3 h. The resultant mixture was diluted with ethyl acetate and then, the organic layer was washed with water, 1 M HCl (aq.), and $NaHCO_3$ saturated aqueous solution, repeatedly. The resultant organic layer was dried over Na_2SO_4, filtrated, and evaporated to give the product as pale yellow solid (0.69 g, 99%). ^{1}H NMR (DMSO-d_6, TMS): 10.00 (s, 2H), 7.63 (d, $J = 8.64$ Hz, 4H), 7.52 (d, $J = 8.52$ Hz, 4H), 2.61 (m, 2H), 1.10 (d, $J = 6.84$ Hz, 12H); IR (KBr, cm^{-1}) 3305, 2150, 1667, 1526; $[M + H]^+ = 373.18$ (calc. 373.18).

1,4-Bis(*p*-(*S*)-2′-methylbutyramidophenyl)butadiyne (DPB-(*S*)Pe). To 1-(*S*)-2′-methylbutyramido-4-ethynylbenzene (0.10 g, 0.48 mmol) in anhydrous pyridine (15 ml) copper(II) acetate (0.18 g, 0.98 mmol) was added and then, the resultant mixture was stirred at 50 °C for 3 h. The resultant mixture was diluted with ethyl acetate and then, the organic layer was washed with water and 1 M HCl (aq.) repeatedly. The resultant organic layer was dried over Na_2SO_4, filtrated, and evaporated to give the pure product as pale yellow solid (75 mg, 75%). ^{1}H NMR (DMSO-d_6, TMS): 10.1 (s, 2H), 7.68 (d, $J = 8.64$ Hz, 4H), 7.52 (d, $J = 8.70$ Hz, 4H), 2.40 (m, 2H), 1.61 (m, 2H), 1.39 (m, 2H), 1.08 (d, $J = 6.84$ Hz, 6H), 0.86 (t, $J = 7.44$ Hz, 6H); IR (KBr, cm^{-1}) 3298, 2961, 2144, 1668, 1580, 1506; $[M + H]^+ = 401.22$ (calc. 401.14).

Preparation of SPG–DPB complexes

s-SPG ($M_w = 150$ kDa) in DMSO (5 mg ml^{-1}, 10 μl) was mixed with DPBs in DMSO (5 mg ml^{-1}, 10 μl) and then, the resultant DMSO solution (20 μl) was diluted with an additional DMSO (180 μl). The resultant DMSO solution (200 μl) was mixed with water (200 μl) to give aqueous DMSO containing 50 v/v% of water. This solution still has high DMSO content and, therefore, DPDs should be loosely accommodated within the partially renatured helical structure of SPG. The resultant solutions were then subjected to a sonication (bath-type, *ca.* 5 min) followed by mixing with additional water (266 μl) to give DPBs in aqueous DMSO (water contents are 70 v/v%).

The referential solution using t-SPG was prepared as follows. s-SPG in DMSO (5 mg ml^{-1}, 10 μl) was diluted with DMSO (180 μl) followed by mixing with water (200 μl). After a sonication, the resultant aqueous DMSO ($[H_2O] = 50$ v/v%) was diluted with water (266 μl) and aqueous DMSO ($[H_2O] = 70$ v/v%, 1334 μl) followed by incubation at room temperature for overnight to ensure entire renaturing of SPG. Finally, DPBs in DMSO (5 mg ml^{-1}, 10 μl) was added into the resultant solution.

Photo-mediated polymerization of SPG–DPB complexes

Photo-mediated polymerization of SPG–DPB complexes was carried out by using UVL-100P (Riko Kagaku Sangyo Co. Japan) with a distance of 5 cm from the samples.

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