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β-1,3-Glucan Polysaccharide (Schizophyllan) Acting as a One-Dimensional Host for Creating Supramolecular Dye Assemblies

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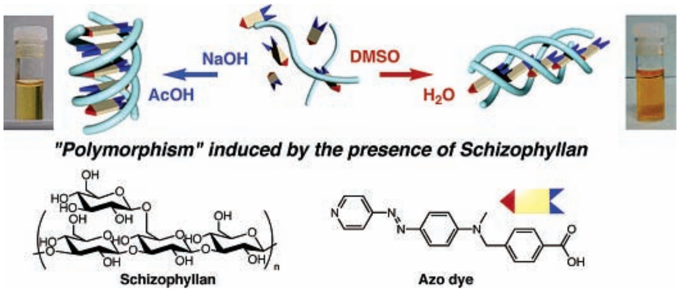
ABSTRACT



We have demonstrated that one-dimensional supramolecular dye assemblies with a uniform diameter can be created by utilizing schizophyllan (SPG) as a one-dimensional host. In the supramolecular nanofibers, the dye molecules are assembled into the different aggregation modes depending on the preparation procedures. The findings establish that SPG is useful for creating the supramolecular nanofibers, where temporal superstructures can be stabilized by the SPG-specific helical higher-order structure.

Dipolar dyes and their one-dimensional assemblies have been of much concern because of their strong absorption band around the visible wavelength region due to the push−pull mechanism of delocalized electrons. These dipolar dyes have inherent properties to form self-assembling architectures through intermolecular $\pi-\pi$ stacking or dipolar−dipolar interactions. So far, creating well-regulated supramolecular assemblies from a rationally designed dye molecule has attracted widespread interest in light of their potential applications for nonlinear optical and photorefractive devices.[1] A particularly challenging aspect is to create a wide variety of supramolecular assemblies from a single dye, for which the rational control of intermolecular dye interactions is considered to be a key process. Nevertheless, only a few attempts have been reported to create a variety of dye assemblies, and the rational control of dye interactions in

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(1) (a) Würthner, F.; Yao, S.; Beginn, U. *Angew. Chem., Int. Ed.* **2003**, *42*, 3247. (b) Parins, L. J.; Thalacker, C.; Würthner, F.; Timmerman, P.; Reinhoudt, D. N. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10042. (c) Jones, R. M.; Lu, L.; Helgeson, R.; Bergstedt, T. S.; McBranch, D. W.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 14769. (d) Yao, S.; Beginn, U.; Gress, T.; Lysetska, M.; Würthner, F. *J. Am. Chem. Soc.* **2004**, *126*, 8336. (e) Morikawa, M.-a.; Yoshihara, M.; Endo, T.; Kimizuka, N. *J. Am. Chem. Soc.* **2005**, *127*, 1358.

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the molecular level seems to still be very difficult. One of the effective ways to overcome this difficulty is to use an appropriate template,[2] such as a biopolymer with a unique higher-order structure, on which dye molecules can be arranged in a well-regulated fashion to generate a wide variety of colors as well as functions, reflecting their original shapes and electronic properties.

Schizophyllan (SPG) is a natural polysaccharide produced by the fungus *Schizophyllum commun* and adopts a triple helix (t-SPG) in nature, which can be dissociated into a single chain (s-SPG) by dissolving in dimethylsulfoxide (DMSO) or alkaline media (Figure 1).[3] The s-SPG chain can retrieve the original triple helix by exchanging DMSO for water or by neutralization of an alkaline solution with an acid. Recently, we have found that when this renaturing process is carried out in the presence of a hydrophobic polymer, the polymer can be incorporated into a one-dimensional cavity created by the SPG helical structure with the aid of hydrophobic interaction to give a clear aqueous solution. This result suggests that SPG can act as a one-dimensional host for a hydrophobic polymer to create a water-soluble nanocomposite, in which the individual guest polymer is isolated and twisted by reflecting the original chiral motif of SPG.[4] One may consider, therefore, that if dye molecules, which are rationally designed to form the self-assembling structures, can be entrapped in the SPG cavity it follows that insulated supramolecular nanofibers with a uniform diameter and an intrinsic helical supramolecular structure can be created. Here, we report our novel findings that the creation of dye assembling structures with a one-handed helical superstructure can be achieved in the unique one-dimensional SPG cavity, where SPG acts not only as a one-dimensional host for dye assemblies but also as a sheath to stabilize the temporarily formed supramolecular polymers.

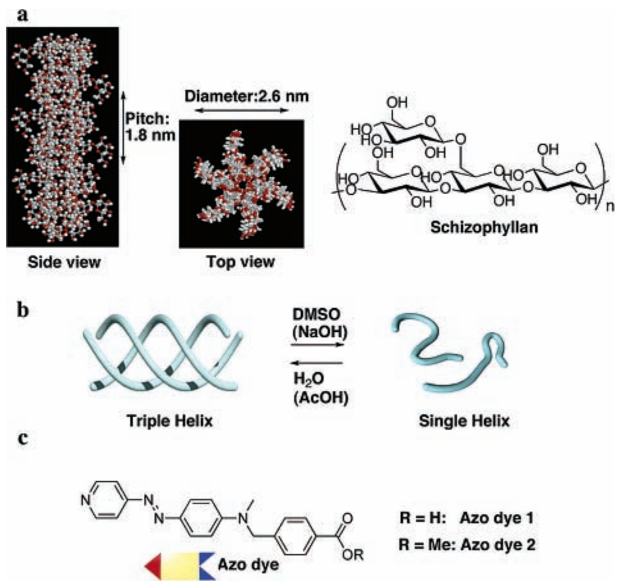

Figure 1. (a) Calculated models of an SPG triple helix and repeating units of SPG. (b) Renature and denature processes of SPG. (c) Designed dipolar dye with pyridine and carboxylic acid terminals.

We designed here a dipolar dye **1** (Azo dye **1**) having pyridine and carboxylic acid terminals (Figure 1c).[5] It is expected that the intermolecular interactions among dye **1** molecules would involve cooperative $\pi-\pi$ stacking and dipolar−dipolar interactions and be mainly dominated by the strong hydrogen-bonding interaction between the terminal groups. This molecular design implies, therefore, that if the composite formation is carried out in the presence of s-SPG utilizing the different renaturing solvents, e.g., a DMSO or NaOH solution, the dye assemblies with different molecular arrangements would be entrapped in the SPG cavity; that is, in DMSO solution, a self-assembling structure would be more dominated by the hydrogen-bonding interaction, whereas in an alkali solution, $\pi-\pi$ stacking and dipolar−dipolar interactions in addition to hydrophobic interactions would become major driving forces.

First, to test the feasibility of this idea, we prepared the DMSO solution containing s-SPG (10.0 mg mL^{-1}, 100.0 μL, MW = 15 000) and mixed it with a DMSO solution of dye **1** (1.0 mg mL^{-1}, 100.0 μL). To the resultant DMSO solution, water (1800 μL) was gradually added to give a clear orange solution. The final composition of water/DMSO (v/v) was adjusted to 95:5 (v/v). During this procedure, the renaturing of s-SPG and the self-assembling of dye **1** through the intermolecular hydrogen-bonding interactions proceeded and the supramolecular dye assemblies thus constructed would be insulated in the SPG one-dimensional cavity. After leaving the resultant solution for 2 days at room temperature, the composite was characterized by spectroscopic and microscopic measurements.

Figure 2a shows UV−vis spectra of the SPG/dye **1** solution. The absorption maximum of dye **1** itself appears at 446 nm,[6] whereas the peak of the composite is red shifted to 468 nm. This significant red shift suggests that *J*-type assemblies are mainly formed in the solution, promoted by

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[5] Similar molecular designs have already been reported: (a) Aoki, K.; Nakagawa, M.; Ichimura, K. *J. Am. Chem. Soc*. **2000**, *122*, 10997. (b) Aoki, K.; Nakagawa, M.; Seki, T.; Ichimura, K. *Chem. Lett*. **2002**, 378. (c) Nakagawa, M.; Ishii, D.; Aoki, K.; Seki, T.; Iyoda, T. *Adv. Mater*. **2005**, *17*, 200.

[6] We took UV−vis spectra of Azo dye **1** as a function of concentration (concentration range: 0−0.1 mg mL^{-1}). From an LB plot at 446 nm, it is confirmed that no aggregate is formed at the concentration range used.

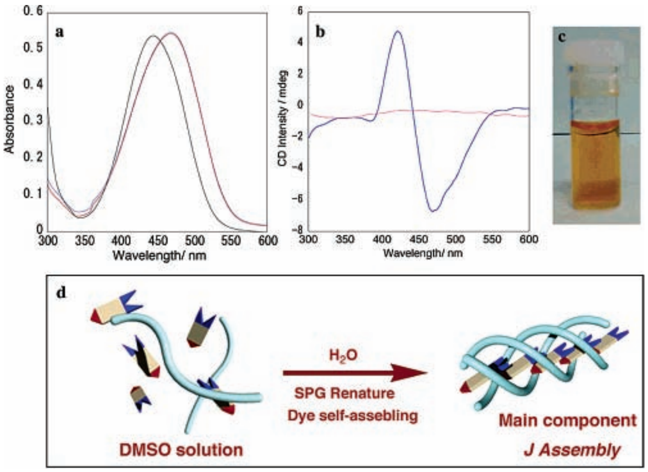

Figure 2. (a) UV−vis and (b) CD spectra of the samples containing the SPG/Azo dye **1** composite (blue lines), monomeric Azo dye **1** in DMSO (black line), and an Azo dye **1** aggregate in a water/DMSO mixed solvent (red lines), prepared from a DMSO solution (1.0 cm, rt). (c) Photo image of the solution containing the SPG/Azo dye **1** composite. (d) Schematic illustration of the *J*-type assembly formation during the renaturing of s-SPG: this type of assembly would be created as a major component in the solution.

the intermolecular hydrogen bonding in addition to $\pi-\pi$ stacking interactions among dye **1** molecules.[7] As a reference experiment, a DMSO solution containing dye **1** itself was diluted with water. Although the mixture provided the precipitate after several minutes, one could measure the UV−vis spectra of the temporarily "transparent" solution, which gave a red shifted absorption maximum (468 nm) comparable with that of the SPG/dye **1** composite. The result indicates that dye **1** itself has the inherent property to form a *J*-type assembly with the aid of hydrogen-bonding interactions even in the absence of SPG. From these results, one may expect that if the *J*-type assembly is entrapped in the SPG cavity it adopts the one-dimensional and helical structure which is enforced by the SPG-specific helical superstructure.

CD spectroscopy is helpful for monitoring the definitive interaction between SPG and dye **1**. Dye **1** itself is optically inactive, so that no CD signal was detected even after forming the *J*-type assembly in the mixed solvent. Upon mixing with s-SPG, however, an intense split-type induced CD (ICD) appeared in the $\pi-\pi^*$ transition region of the dye **1** assembly (Figure 2b). The result strongly supports the view that the *J*-type assembly is entrapped in the one-dimensional cavity, in which the self-assembling nanofiber structure with the twisted molecular arrangement is created through the intermolecular hydrogen-bonding interaction among dye **1** molecules.[9]

As an alternative strategy, we tried to prepare the composite utilizing the neutralization process of an alkaline solution containing SPG and dye **1**, expecting that the different dye arrangement may be achieved through $\pi-\pi$ stacking and dipolar−dipolar interactions. It is known that the triple strand of SPG is dissociated into the single strand at pH > 12, whereas it retrieves the original triple strand by pH neutralization.[3] Accordingly, we prepared an NaOH solution containing s-SPG (10.0 mg mL^{-1}, 100.0 μL, pH = 13) and mixed it with an NaOH solution of dye **1** (1.0 mg mL^{-1}, 100.0 μL, pH = 13), where the carboxylic terminal group should be dissociated into the carboxylate anion at this stage. To the resultant solution, AcOH was gradually added to give a clear yellow solution. The final pH was adjusted to 7.0. UV−vis and CD spectroscopic measurements of the resultant solution revealed that the self-assembling architecture of dye **1** is created in the SPG one-dimensional cavity. As shown in Figure 3a, one can recognize that the absorption maximum of dye **1** is blue shifted from 446 to 417 nm, accompanied by a slight peak broadening. This peak broadening implies that the obtained yellow solution contains several unidentified aggregates as minor components. However, taking the blue shift of the absorption maximum into consideration, we can propose that the main structure created by dye **1** is an *H*-type assembly, in which dye **1** adopts the antiparallel orientation through dipolar−dipolar interactions.

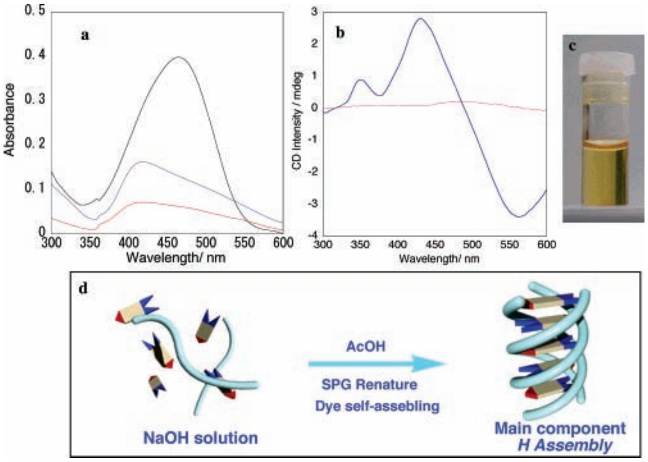

Figure 3. (a) UV−vis and (b) CD spectra of the samples containing the SPG/Azo dye **1** composite (blue lines), monomeric Azo dye **1** in DMSO (black line), and the Azo dye **1** aggregate in a water/DMSO mixed solvent (red lines), prepared from NaOH solution (1.0 cm, rt). (c) Photo image of the solution containing the SPG/Azo dye **1** composite. (d) Schematic illustration of the *H*-type assembly formation during the renaturing of s-SPG: this type of assembly would be created as a major component in the solution.

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[7] The formation of the intermolecular hydrogen-bonding interaction is directly evidenced by IR spectroscopy (Supporting Information, Figure S1). The stretching band of the pyridine group appears at around 1371 cm^{-1}, whereas after mixing SPG, the peak is shifted to 1379 cm^{-1}. For related papers, see: Tanaka, S.; Shirakawa, M.; Kaneko, K.; Takeuchi, M.; Shinkai, S. *Langmuir* **2005**, *21*, 2163 and references cited therein.

[8] Similar morphologies were confirmed in TEM images (Supporting Information, Figure S4).

[9] As the CD signal shows the negative Cotton effect, one may consider that the left-handed helical structure is created inside the SPG cavity despite the right-handed helical nature of SPG. The CD sign reflects the dye dipole moments in the microscopic level, whereas the morphology reflects the aggregation mode in the macroscopic level. These two factors are not necessarily correlated. In fact, such a mismatch has been observed by Würthner et al.[1c]

As a reference experiment, we carried out the sample preparation in the absence of SPG. When AcOH was added to the NaOH solution containing dye **1**, a yellow precipitate was gradually formed with decreasing pH values from 13 to 7. Although the absorbance of dye **1** largely decreased because of this precipitate formation, one could still estimate the absorption maximum of this weak band in the supernatant phase: the value, 414 nm, suggests that dye **1** results in the *H*-type assembly even in the absence of SPG. This result supports the view that if the self-assembling structure of dye **1** is prepared in the presence of s-SPG it will be entrapped in the SPG cavity to give the water-soluble composite. Furthermore, as shown in Figure 3b, ICD is also detected at the $\pi-\pi^*$ transition region of the dye **1** assembly, indicating that the one-dimensional *H*-type assembly is entrapped in the helical SPG cavity. These results clearly support the view that the intermolecular hydrogen-bonding interactions among dye **1** molecules are indispensable for *J*-type assembly formation. This means that the different dye assemblies can be created from dye **1** depending on the preparation procedures, which control the hydrogen-bonding capability of dye **1**. One may regard this phenomenon to be a sort of "polymorphism" induced by the presence of SPG.

To obtain further reliable evidence that the hydrogen-bonding interactions among dye **1** molecules are indispensable for the creation of a *J*-type assembly, we carried out the sample preparation utilizing dye **2** (Figure 1c), which has no ability to form the intermolecular hydrogen bond due to the lack of the carboxylic acid terminal group. The sample was prepared from a DMSO solution according to the same procedure as described above. As shown in Supporting Information Figure S2a, the absorption maximum of the resultant solution is blue shifted to 417 nm as expected for the assembly formation through non-hydrogen-bonding interactions. The result indicates that dye **2** forms *H*-type assemblies even in the water/DMSO mixed solvent, which is in sharp contrast to the *J*-type assembly formation observed for the dye **1** system.

Here, one may consider that the observed spectral difference between *J*- and *H*-type assemblies is attributed to the difference in solvent properties surrounding the composites; that is, the *J*-type assembly is always created in the water/DMSO mixed solvent, whereas the *H*-type assembly is created in water containing AcONa salt formed through the neutralization process. Thus, to compare the spectral features of two composites under the same conditions, we removed DMSO or AcONa utilizing an ultrafiltration technique. UV−vis spectra of the obtained aqueous solutions are shown in Supporting Information Figure S3. It is seen from Figure S3 that the absorption maximum and the spectral features of these samples are not changed even after exchanging the solvents for pure water. Consequently, we can reasonably conclude that the entrapped supramolecular structures in the SPG cavity are not affected by the solvent properties, suggesting that the SPG sheath effectively stabilizes the entrapped one-dimensional supramolecular structures, reflecting their preparation processes.

The intrinsic property of SPG as a one-dimensional host can be well characterized by the morphological images of the obtained composites. Figures 4a−d show the AFM images of SPG/dye **1** composites. In Figures 4a,c, one can recognize many fibrous structures with 10−20 nm height, whereas in Figures 4b,d, dye **1** itself results in an amorphous aggregate mass and did not give any fibrous structures.[8] Taking the diameter of the composites into consideration, somewhat larger *J*- or *H*-type assemblies must be entrapped in the SPG cavity. It should be noted that the amorphous aggregates formed in the bulk phase also contain *J*- or *H*-type assemblies because their UV−vis spectral patterns are almost the same as those of the composites with SPG. The findings establish that SPG strongly restricts formation of the three-dimensional amorphous mass during the self-assembling process of dye **1** and forces the formed assemblies to adopt the one-dimensional structure with a uniform diameter.

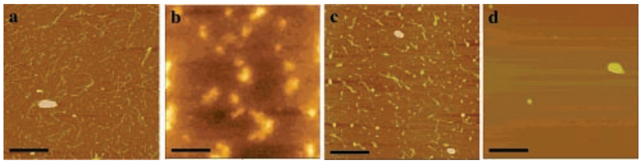

Figure 4. AFM images of the samples (bar = 500 nm): (a) composite with SPG, (b) only Azo dye **1** prepared from a DMSO solution, (c) composite with SPG, and (d) only Azo dye **1** prepared from a NaOH solution. The composite was casted on mica.

In summary, we have demonstrated that *J*- or *H*-type dye assemblies can be selectively created in the SPG cavity by utilizing the two different renaturation processes of s-SPG. The findings clearly show that SPG can act as a unique one-dimensional sheath not only for synthetic polymers as shown in the preceding papers[4c,e] but also for the supramolecular nanofibers as demonstrated in this study, where temporal superstructures can be stabilized inside the cavity by the wrapping effect of SPG. We believe that the present system will provide a novel concept for creation of supramolecular nanofibers and will play an important role in the design of novel optical and photorefractive devices.

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Supporting Information Available: IR spectra of the SPG/Azo dye **1** composite. UV−vis and CD spectra of the SPG/Azo dye **2** composite. Comparison of UV−vis spectra after dialysis. TEM images of the SPG/Azo dye **1** composite. This material is available free of charge via the Internet at http://pubs.acs.org.

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