Nucleotide Bolaamphiphiles

Oligonucleotide-Templated Self-Assembly of Nucleotide Bolaamphiphiles: DNA-Like Nanofibers Edged by a Double-Helical Arrangement of A–T Base Pairs

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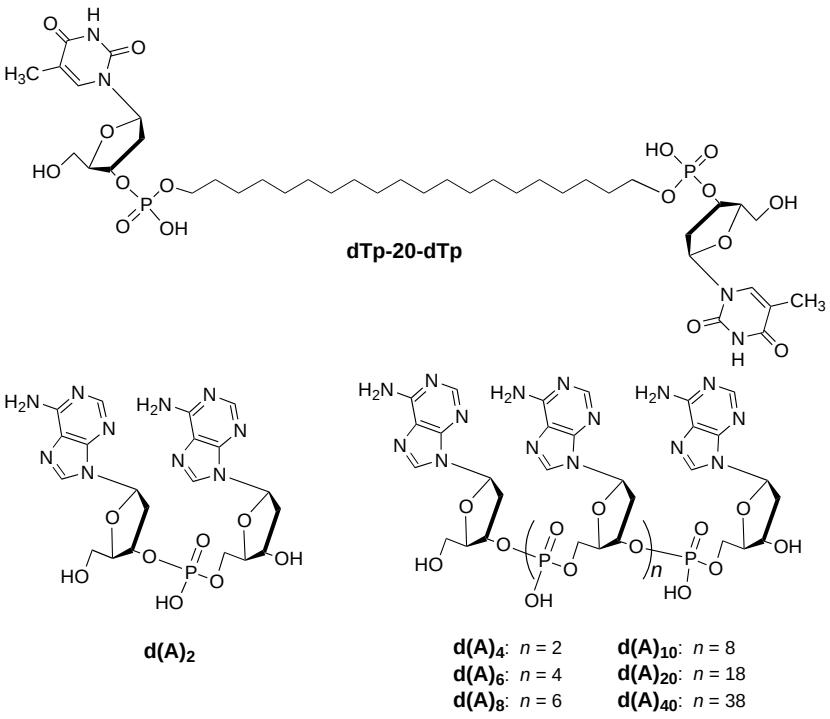
In nanotechnology the "bottom-up" approach, the fabrication of nanostructures from molecular building blocks, is of great interest owing to its ease in preparation, low energy consumption, and potential to produce a great number of molecular nanostructures with well-defined three-dimensional morphologies.[1,2] In particular, self-assembly driven by molecular recognition has been recognized as a powerful strategy to give well-controlled hierarchical supramolecular assemblies[3] as well as hetero-assembled liquid crystals.[4] Recently, the combination of template effects with molecular recognition resulted in the hierarchical generation of supramolecular fibers, in which oligoisophthalamide and cyanuric acid served as the template.[5] The most sophisticated and advanced system based on template-induced and multiple-recognition-controlled fabrication is exemplified by natural DNA. It is, therefore, reasonable to use DNA as a template for making novel structures and highly functionalized materials. Most DNA-templated molecular assembly is, however, based on either the use of complementary DNAs[6] or the use of multiple electrostatic interactions along the DNA backbone.[7] Some researchers have also described DNA-ordered assembly in between lipid membranes by using a cationic lipid and anionic DNA.[8] We report here on the formation of DNA-like nanofibers, which are edged by a double-helical arrangement of A–T base pairs. These nanofibers result from the complementary oligonucleotide-templated self-assembly of the thymidine-appended bolaamphiphile **dTp-20-dTp** with a series of oligoadenylic acids **d(A)$_n$** ($n = 2$, 4, 6, 8, 10, 20, 40) .

The binary self-assembly of **dTp-20-dTp/d(A)$_n$** ($n = 2$, 4, 6, 8, 10, 20, 40) having the same number of adenine and thymine residues was found to gelatinize water after incubation for several days. These hydrogels have very different appearances. With increasing chain length of the template oligoadenylic acid, the color of the hydrogel changes gradually from white to transparent, and also the gel stiffness changes from rigid to loose. The morphology of the self-assembled nanofibers of the thymidine bolaamphiphiles proved to be strongly dependent on the chain length of the complementary oligoadenylic acid template. We recently reported on a TEM study of the hydrogel formed from **dTp-20-dTp** alone, in which we found three-dimensionally intertwined nanofibers 10–30 nm wide and several micrometers long,[9] dimensions typically seen in the gel structures of low-molecular-weight gelators.[10] Now we find that the nanofiber networks arising from the self-assembly of **dTp-20-dTp** in the presence of the relatively short templates **d(A)$_n$** ($n = 2$, 4, 6, 8) are similar to those formed from the single component alone



(Figure 1 a–d). When the chain length of the template is increased ($n = 10$, 20, 40), discrete helical nanofibers become evident, which have a width of 7–8 nm and length of several hundred nm (Figure 1 e, 1 f, and 1 h). From the high-magnification image we estimate a helical pitch of approximately 20 nm (Figure 1 g). Interestingly, the fiber lengths of the binary **dTp-20-dTp/d(A)$_{40}$** self-assembly extend to > 1 μm—much longer than that of the **dTp-20-dTp/d(A)$_{20}$** assembly (150–700 nm). Thus, for the nanostructures obtained with the range of templates from **d(A)$_2$** to **d(A)$_{40}$** we clearly observe both the increasing length of the nanofiber and, for templates with $n \geq 10$, the manifestation of helical structure.

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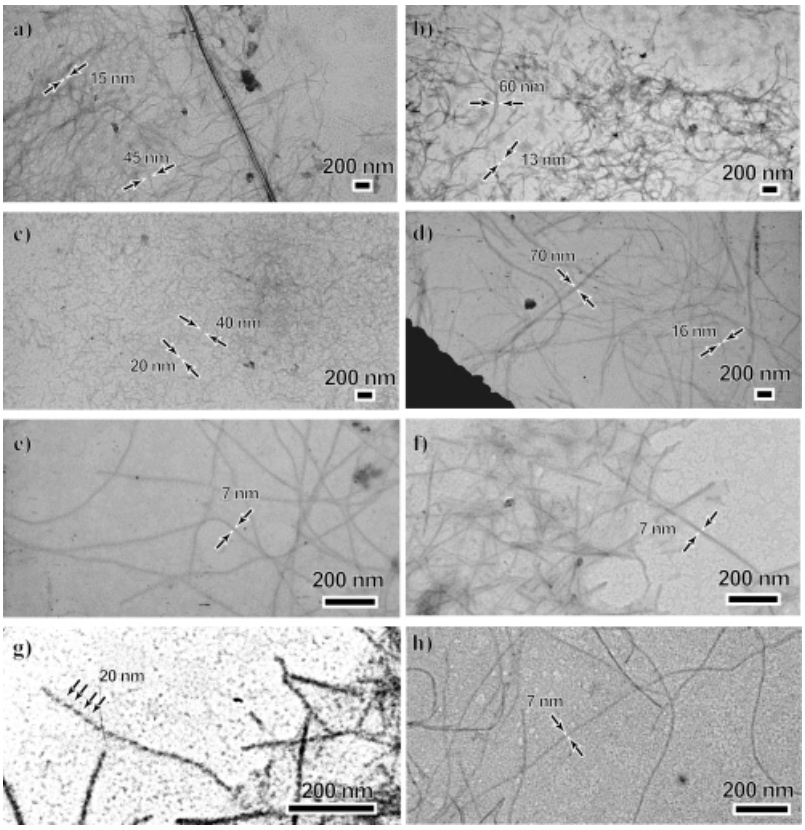

Figure 1. TEM image of nanofibers self-assembled from **dTp-20-dTp** in the presence of a) $\mathbf{d(A)_2}$, b) $\mathbf{d(A)_4}$, c) $\mathbf{d(A)_6}$, d) $\mathbf{d(A)_8}$, and e) $\mathbf{d(A)_{10}}$. TEM image of discrete, relatively long nanofibers self-assembled from **dTp-20-dTp** in the presence of f) $\mathbf{d(A)_{20}}$ and h) $\mathbf{d(A)_{40}}$. g) High-magnification TEM image of discrete supramolecular DNA-like nanofibers in f). The arrows note the helical pitch of 20 nm.

To verify that **dTp-20-dTp** actually complexes with each adenine unit of the oligoadenylic acid in water, we carried out electrospray-ionization Fourier-transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS), which has proved to be a powerful tool for the analysis of weakly bound species.[11] The soft ionization, high resolution, and high sensitivity of ESI-FTICR MS allow us to detect three peaks for **dTp-20-dTp** and $\mathbf{d(A)_2}$ in aqueous solutions, which are attributable to the 1:1 and 2:1 complexes.[12] These findings strongly support the view that **dTp-20-dTp** first complexes with a complementary oligoadenylic acid and then assembles to form nanofibers.

Attenuated total reflectance (ATR)-FTIR spectroscopic studies of each binary self-assembly in D_2O dispersion are quite compatible with this observation. In the case of the single-component **dTp-20-dTp** hydrogel, the thymine carbonyl stretching vibration appears at 1712 cm^{-1}, indicating that the thymine carbonyl groups take part in hydrogen bonding through either the T–T base pair or thymine–water interaction. In contrast, this band completely disappears for the $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{10}}$ hydrogel, indicating the liberation of the thymine carbonyl groups from hydrogen bonding (Figure 2). It should be noted here that the thymine carbonyl groups are free in Watson–Crick and Hoogsteen base-pairing.[13] Thus, this observation can support the fact that the thymidine bolaamphiphile **dTp-20-dTp** forms a complementary complex with $\mathbf{d(A)_{10}}$ at both ends through hydrogen-bonded A–T base pairs. The $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_n}$ ($n = 20, 40$) binary self-assemblies also did not display this carbonyl vibration band. These ESI-FTICR and ATR-FTIR results also exclude the possibility of T-A-T base alignment upon complexation of **dTp-20-dTp** with the comparably longer $\mathbf{d(A)_n}$ ($n = 10, 20, 40$). Eventually the binary assemblies organize to form discrete nanofibers 7 nm wide. On the other hand, the observed nanofiber bundles and branches for $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_n}$ ($n = 2, 4, 6, 8$) may arise from the cross-linking of the shorter oligoadenylic acids with **dTp-20-dTp** through A–T base pairs since the shorter $\mathbf{d(A)_n}$ ($n = 2, 4, 6, 8$) oligonucleotides are too short to act as templates for the formation of single nanofibers.

Variable-temperature circular dichroism (VT-CD) spectroscopy was very useful to get insight into both the enhancement of the helical profile and the melting behavior of the obtained nanofibers depending on the template lengths. Figure 3 displays VT-CD spectra for the binary self-assemblies in aqueous solutions. The CD spectrum of the $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_2}$ assembly looks much different from those of the $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_n}$ ($n = 10, 20, 40$) assemblies and also displays little change upon heating from 25 °C to 90 °C (Figure 3a). In contrast, the CD spectra of the $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{20}}$ and $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{40}}$ assemblies are temperature dependent (Figures 3b and 3c). The CD spectral profiles for the series of binary self-assemblies $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{20}}$ ($n = 10, 20, 40$) quite resemble that of **poly(dA)-poly(dT)** B-DNA,[14] in which the two strands of the sugar–phosphate backbone are stabilized by A–T base pairs. Furthermore, the temperature dependence of the CD intensities evaluated at $\lambda = 250$ nm shows a drastic increase at approximately 50 °C for $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{20}}$ and at 60 °C for $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{40}}$ (Figure 3b and 3c, inset). These observed T_m values are comparable to the previously measured T_m ($T_m = 53$ °C under the condition of $[Na^+] = 0.02$ M)[15] of the **poly(dA)-poly(dT)** B-DNA, which

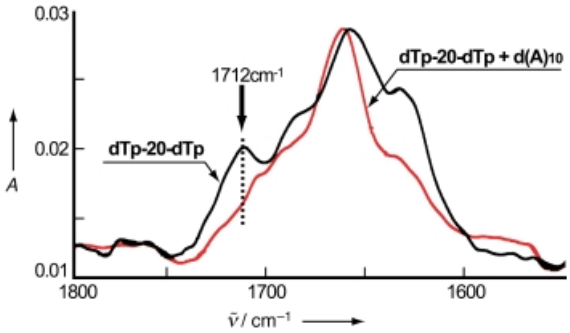

Figure 2. ATR-FTIR spectra of the **dTp-20-dTp** monoassembly and the $\mathbf{dTp\text{-}20\text{-}dTp/d(A)_{10}}$ binary assembly in D_2O. A = absorbance.

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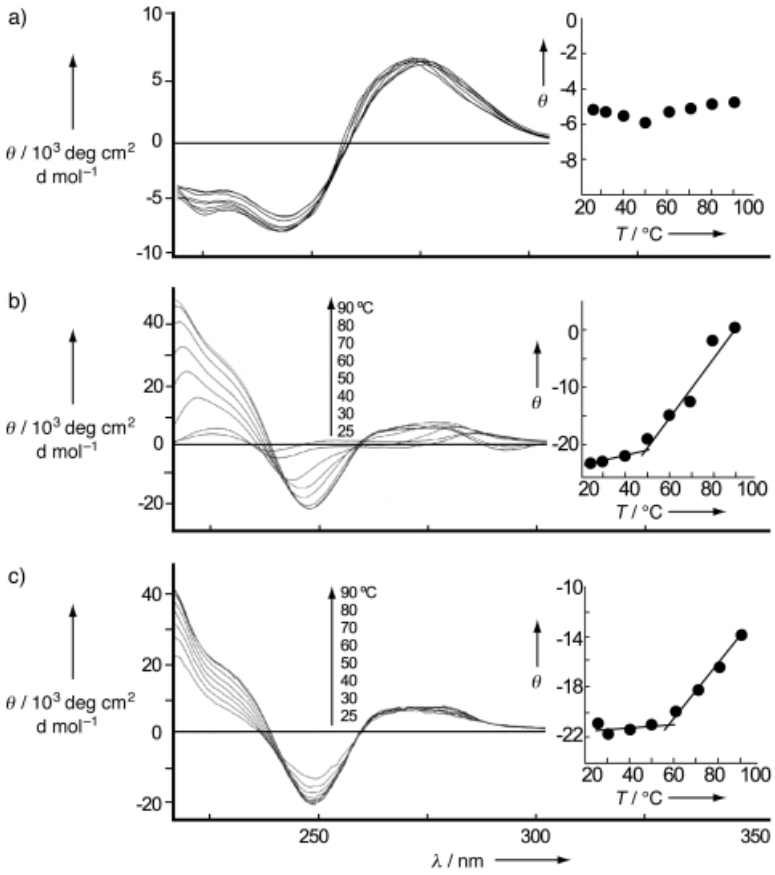

Figure 3. CD spectra of highly dilute aqueous solutions of the binary assemblies a) **dTp-20-dTp/d(A)$_2$**, b) **dTp-20-dTp/d(A)$_{20}$**, and c) **dTp-20-dTp/d(A)$_{40}$**, which were recorded with a 0.01-cm quartz cell ($c = 4 \times 10^{-3}$ M). Each inset shows the temperature dependence of the CD intensities at $\lambda = 250$ nm. θ = molar ellipticity.

proves directly the dissociation of the A–T base pairing. At this time, the possibility of the T-A-T base pair alignment can also be excluded since the concentration of divalent cations is very low and the pH is close to neutral in this system. One may notice here the relatively high thermal stability for the **dTp-20-dTp/d(A)$_{40}$** assembly. Thus, the template effect of the oligoadenylic acids on the self-assembled morphologies becomes evident with increasing chain length. In addition, longer templates **d(A)$_n$** ($n = 10$, 20, 40) are able to induce helical morphologies in single nanofibers, which are absent in the homo-assembled nanofibers.

Considering all of data and evidence presented here, one may envisage that the complementary A–T base pairs are aligned helically, edging both ends of the bolaamphiphile. Double strands of the oligonucleotide wind around the vertically stacked rods of the bolaamphiphiles around the same axis to give helical nanofibers (Figure 4). The CH_2 symmetric and antisymmetric stretching bands appear at 2918 and 2845 cm^{-1}, respectively, indicating a highly populated all-*trans* conformation on the oligomethylene chain. The center-to-center distance between two neighboring phosphate moieties of poly(adenylic acid)s is known to be approximately 0.7 nm in B-DNA, which fixes the spatial arrangement of two neighboring thymine residues of the stacked bolaamphiphiles at rotation angles of 8–10°. Thus, 36–45 of the thymidine bolaamphiphiles will be maximized at helical pitches of about 20 nm. This situation allows the base pair to be in the hydrophobic environment. Thus, the hydrophilic moieties of deoxyribose and phosphodiester of **dTp-20-dTp** are successfully exposed to external aqueous dispersion. The top view of the schematic illustration of molecular packing clearly shows this convenient circumstance for nanofiber formation (Figure 4). On the basis of the similarity of the CD spectra to that of B-DNA, the A–T base stacking is considered to form right-handed helical structures.

The diameter of the nanofiber obtained (7–8 nm) is slightly greater than the length of extended **dTp-20-dTp** molecules (3.5 nm). The double-helical strands of the sugar–phosphodiester backbone chains (two species, each 1.8–2.0 nm wide) of oligoadenylic acid are edging each end of the bolaamphiphile. Therefore, the measured widths of the nanofibers are well compatible the proposed structure.

In summary, we have demonstrated the formation of DNA-like nanofibers that can be seen in TEM through complementary oligonucleotide-templated self-assembly. This methodology should aid in accurately tuning the dimensions of high-axial-ratio nanostructures.

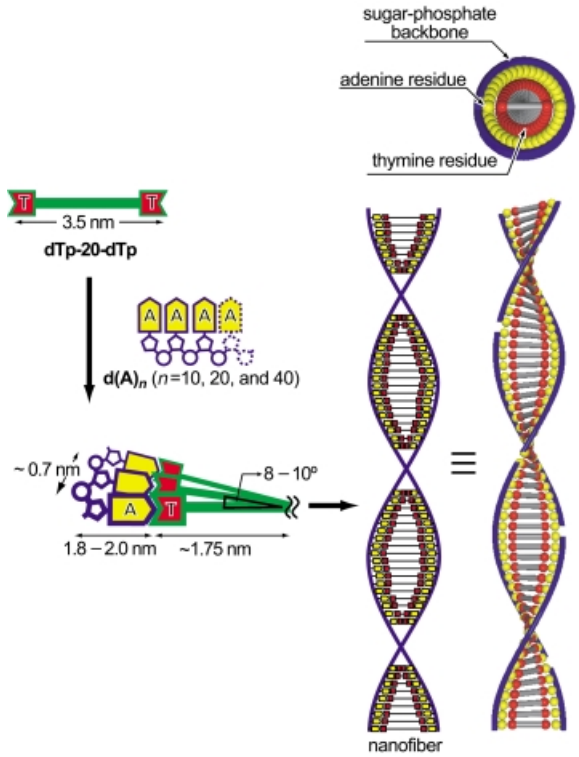

Figure 4. Illustration of the proposed mechanism of formation of the oligoadenylic acid templated self-assembly of the thymidine bolaamphiphiles. Red and yellow units represent the thymine and adenine residues, respectively.

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Experimental Section

The thymidine bolaamphiphile **dTp-20-dTp** was synthesized by using the phosphoramidite technique as described in reference [9]. Bolaamphiphile **dTp-20-dTp** was dispersed in water and completely dissolved by heating at 60 °C and sonication. To this aqueous solution was added an aqueous solution of $\mathbf{d(A)_n}$ ($n = 2, 4, 6, 8, 10, 20, 40$). For all the thymine residues to complex with adenine residues in a 1:1 ratio, the concentrations of **dTp-20-dTp** and $\mathbf{d(A)_{40}}$ were, for example, adjusted to 2.2×10^{-2}, 4.4×10^{-2}, 2.2×10^{-3}, and 1.1×10^{-3} M, respectively. TEM was carried out with a LEO 912 instrument (acceleration voltage 120 kV, Carl Zeiss). CD studies were performed on a JASCO J-800 spectropolarimeter operating between 220 and 400 nm.

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