Two-Dimensional Densely Packed DNA Nanostructure Derived from DNA Complexation with a Low-Generation Poly(amidoamine) Dendrimer

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One of the keys for using deoxyribonucleic acid (DNA) as a nanomaterial relies on how the individual DNA chain can be aligned and how a multitude of DNA chains can be packed into ordered nanostructures. Here we present a simple method for constructing a 2-D densely packed DNA nanostructure using the electrostatic complex of DNA with a poly(amidoamine) (PAMAM) dendrimer of generation two. Ordered DNA arrays are formed by drop-casting an aqueous solution containing positively overcharged complexes onto mica followed by a prolonged incubation. During the incubation, the complexes tend to adsorb onto the negatively charged mica surface through electrostatic attraction. The rodlike complexes organize to form ordered arrays to increase the surface density of the adsorbed complexes and hence the attractive free energy of adsorption. The densely packed nanostructure obtained here is distinguished from the previously reported spheroid or toroid structure derived from DNA complexations with the higher-generation dendrimers.

Introduction

Manipulating the assemblies of molecules on the nanometer scale to generate ordered nanostructures is an important task in the development of the newest generation of materials. Because many biomolecules have specific binding properties for self-assembly, they have been considered to be attractive building blocks for producing controllable nanostructures. Deoxyribonucleic acid (DNA) in particular has received significant attention as a molecular scaffold for nanotechnology[1] and self-assembled nanostructures.[2] DNA has a special double-helix conformation with a diameter of ca. 2 nm and a widely variable length. The π-electron core of the bases stacking along the duplex backbone makes DNA a good candidate for long-distance and 1-D electron transport.[3] DNA-based nanotechnology is a vibrant and expanding field. In recent years, DNA has been widely studied as a material for DNA-based computation[1,4−6] and conducting nanowires.[7]

DNA chains in aqueous media are semirigid with a persistent length of ca. 50 nm;[8] therefore, a long DNA chain is coil-like on a global length scale with its end-to-end distance being smaller than the contour length. One of the keys in using DNA as a nanomaterial relies on how the individual DNA chain can be aligned and how a multitude of DNA chains can be packed into ordered nanostructures. Various physical methods including molecular combing,[9] electrophoretic stretching,[10] and hydrodynamic stretching[11] have been developed to orient DNA on a solid support. Though these methods are effective for stretching the individual chains,[12] they are not plausible for producing densely packed DNA arrays, which are of interest for the developments of photonic materials,[13] biochips,[14] and scaffolds for the assembly of molecular electronic components.[15]

Here we report a method for creating a 2-D densely packed DNA nanostructure via the electrostatic complexation of DNA with a dendrimer. The present approach enables DNA to be fixed by the dendrimer molecules on a mica substrate without prior modification of the substrate surface. A dendrimer is a type of hyperbranched macromolecule composed of layers of monomer units radiating from a central core. Each complete grafting cycle is called a generation.[16,17] The amine groups of a poly(amidoamine) (PAMAM) dendrimer with an ethylenediamine (EDA) core can be positively charged through proton transfer in acidic aqueous media. The resultant cationic dendrimers can then be mixed with polyanionic DNA to form an electrostatic complex. Previous studies have shown that condensed mesophases with the DNA chains surrounded by the dendrimer molecules organized in a square or hexagonal lattice were formed in the bulk solution through complexation.[18,19] This demonstrates that the complexation induces the aggregation of DNA to form an ordered structure, a phenomenon known as DNA condensa-

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tion.[20,21] Here we focus on the use of positively overcharged complexes of DNA with a PAMAM dendrimer of generation two (G2) to create a 2-D densely packed DNA nanostructure on a mica substrate. It will be shown that the adsorption of these complexes on mica through electrostatic attraction can result in ordered DNA arrays. This ordered 2-D nanostructure is uniquely different from the spheroid[22] or toroidlike[22] DNA condensation reported earlier for DNA complexations with higher-generation PAMAM dendrimers[22,23] or other condensing agents.[24]

Experimental Section

Linear DNA-type XIV from herring testes sodium salt (Na content 6.2%) was purchased from Sigma and used without further purification. Its molecular weight as determined by gel electrophoresis was found to have a polydisperse value between 400 and 1000 base pairs (bp) with a center of distribution at ca. 700 bps.[25] PAMAM G2 dendrimer with an EDA core in methanol solution was acquired from Aldrich. After thorough drying, the solid was redissolved in distilled water to produce a 0.2% (w/v) stock solution.

For the preparation of the DNA/PAMAM G2 complex, 5.32 mg of 20 mg/mL DNA aqueous solution was first added to 10 mg of dendrimer solution. The molar ratio of the amine groups of the dendrimer to the phosphate groups of DNA (x) prescribed by this feed ratio was 5.6. Distilled water was subsequently added to reduce the DNA concentration to 4 mg/mL. The protonation of the dendrimer in the solution by 0.1 N HCl(aq) was carried out by potentiometric titration using an Istek desktop pH/mV/TEMP meter (model 720P) equipped with a micro-pH electrode at 25 °C. The titrant was added dropwise in 25 μL portions using a micropipet until the solution achieved a constant pH of 5.2. More distilled water was then added to the solution, and the above potentiometric titration was repeated until the DNA concentration in the solution reached 2.0 mg/mL. The complexation between DNA and the dendrimer took place spontaneously during the titration, as manifested by visually observable precipitation. Finally, a system consisting of the primary complex in the precipitate and the positively overcharged complex in the supernatant was obtained. The overall concentration of the complex in the aqueous media was 5.8 wt % (or the water content was 94.2 wt %).

AFM experiments were performed with a Seiko SPA-300HV scanning probe microscope operated in noncontact mode. Nanosensors SuperSharp silicon tips with a force constant of 15 N/m and a resonance frequency of 130 kHz were used for the measurements.

Results and Discussion

The PAMAM G2 dendrimer contains 30 amine groups, with 16 being the surface primary amine group and the other 14 being the inner tertiary amine group. Both types of amine groups can be protonated at pH 5.2.[26] At $x = 5.6$, the total number of positively charged ammonium groups is in excess of that of the negatively charged phosphate groups; therefore, the complex is located in the positively overcharged regime where the complexation leads to the coexistence of the primary complex in the precipitate with the overcharged complex remaining well dispersed in the supernatant, as illustrated in Figure 1a. The amount of dendrimer bound to the DNA chains in the overcharged complex is more than that required to neutralize the charges on DNA.[27,28] This overadsorption is driven by the entropic gain from the counterion release;[29,30] more specifically, the adsorption of additional dendrimers onto the outer surface of the primary complex having attained optimum charge matching in the interior would release into the bulk solution a certain number of the original Cl^- counterions condensed onto the dendrimers.[25] The overcharged complexes remain well dispersed in the supernatant because the size of their aggregates is limited by their mutual repulsion.

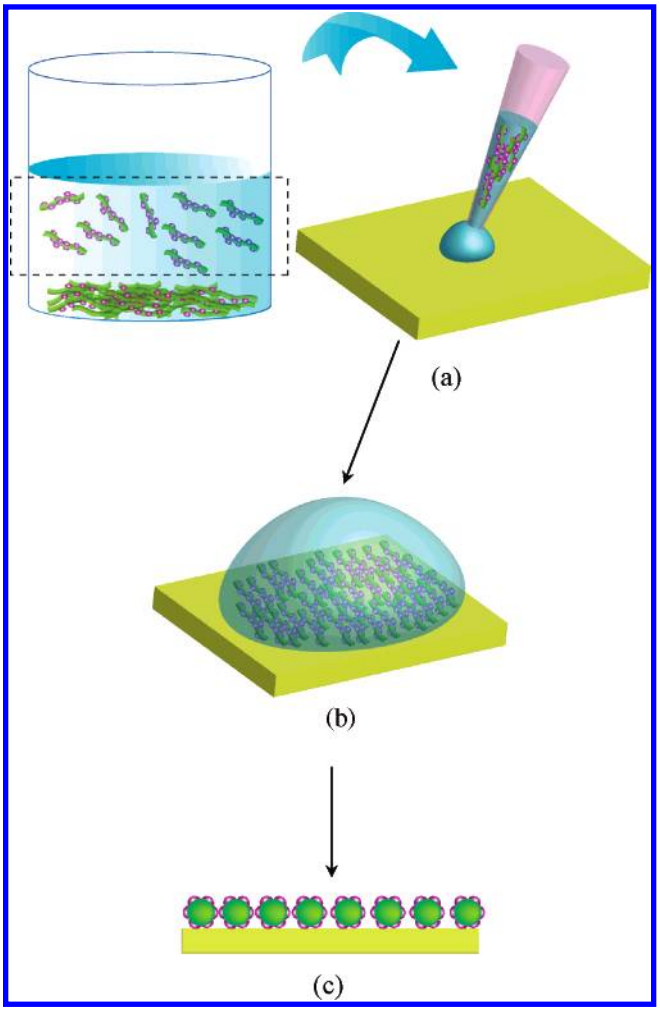

Figure 1. Schematic illustration of the preparation of ordered DNA arrays on a mica substrate using a DNA/PAMAM G2 complex. (a) Drop-casting the supernatant containing the positively overcharged complexes onto the mica substrate; (b) incubation at 4 °C for more than 12 h to allow the adsorption of the overcharged complexes onto the slightly charged mica substrate through electrostatic attraction; (c) drying at room temperature under the ambient atmosphere to obtain the 2-D-ordered DNA arrays in the dry state.

Figure 2 compares the UV−vis spectrum of the supernatant with that of bare DNA to verify the existence of the positively overcharged complex in the supernatant. Bare DNA exhibits an absorption band at 260 nm, whereas pure dendrimer does not show any detectable absorption in the wavelength range of 240−400 nm. The supernatant is found to display an absorption at a higher wavelength of ca. 277 nm. The red shift of the UV absorption shows that the DNA chains in the supernatant of the

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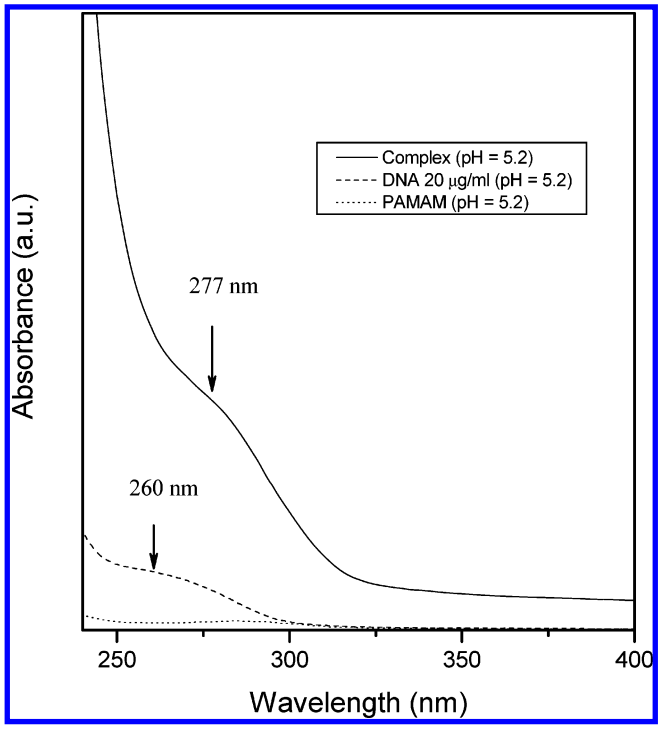

Figure 2. UV spectra of the suprenatnat of a DNA/PAMAM G2 dendrimer complex with $x = 5.6$, a bare 20 μg/mL DNA solution (pH 5.2), and a pure 50 μg/mL PAMAM G2 dendrimer solution (pH 5.2). Bare DNA exhibits an absorption near 260 nm (---), whereas the bare PAMAM dendrimer does not show detectable absorption in the range of 240−400 nm (•••). The supernatant of the complex displays a band at a higher wavelength of ca. 277 nm (−), showing that the DNA is electrostatically bound with the dendrimer to form the positively overcharged complex.

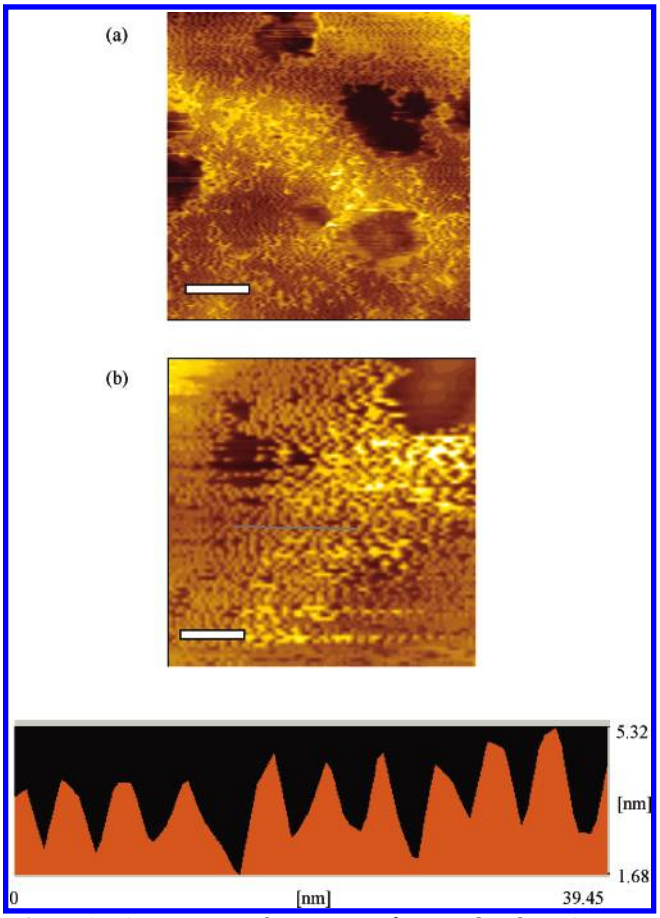

Figure 3. AFM topographic images of 2-D-ordered DNA arrays on a mica surface in air. (a) Lower magnification with the scale bar representing 1 μm and (b) higher magnification and the section profile. The scale bar corresponds to 20 nm.

DNA/dendrimer system do bind (electrostatically) with the dendrimers to form the overcharged complex.[31]

The procedure for producing an ordered DNA structure on a mica substrate using the positively overcharged complex in the supernatant is schematically illustrated in Figure 1. A droplet (~200 μL) of the supernatant solution was applied to cover the whole surface of a freshly cleaved mica substrate (1 cm × 1 cm), and the system was then incubated at 4 °C for more than 12 h. During the incubation, the rodlike overcharged complexes adsorbed onto the slightly negatively charged mica surface as a result of electrostatic attraction. As more and more complexes condensed on the substrate, they had to organize into ordered arrays to allow more adsorption of the complexes. For polyelectrolytes strongly interacting with an oppositely charged surface, it has been shown that ordered phases of the adsorbed polyelectrolytes will be formed to increase the surface density of the adsorbed polyelectrolyte chains to reduce the free energy associated with the electrostatic interaction and excluded volume of the system.[32] Once this free-energy reduction overwhelms the loss of the translational and conformational entropy of the polyelectrolytes upon adsorption, the formation of the ordered phase is favored.

Figure 3 displays the AFM topographic images of the DNA/dendrimer complex condensed on the mica surface. (See Supporting Information for additional micrographs of other places in the same sample.) It can be seen that DNA chains with a significant degree of positional and orientational order form ordered arrays on the surface. The average interhelical distance in the arrays estimated from the section profiles is about 3.8 nm. Previous studies have reported that the measured height of a single DNA chain adsorbed on mica was always less than the actual diameter of DNA (~ 2 nm) as a result of sample deformation by tip interaction, dehydration of the molecule, and salt deposition.[33] In this case, the height measurement of DNA in air using tapping mode AFM usually revealed a value of about 0.7 nm.[33,34] The height of the DNA arrays observed in Figure 3 is 2.6 ± 0.2 nm, which is apparently larger than that of a monolayer of bare DNA. Because the formation of multiple layers of rodlike complexes on the substrate is not plausible because the strong repulsion between the overcharged complexes should prohibit the disposition of another layer of the complexes onto the already adsorbed monolayer, the additional height is considered to be contributed by the dendrimer molecules bridging a monolayer of DNA with the mica surface. It is also noted that the close packing of the overcharged complexes may exert a repulsive force to resist the deformation of the DNA chains induced by the tip interaction and hence increases the observed height compared with that of bare DNA.

Fang and Yang have found similarly ordered DNA arrays formed by the 2-D condensation of DNA on the supported cationic lipid membranes in the hydrate state.[35,36] Rau et al. showed that such a DNA array structure is stabilized by a hydration force;[37]

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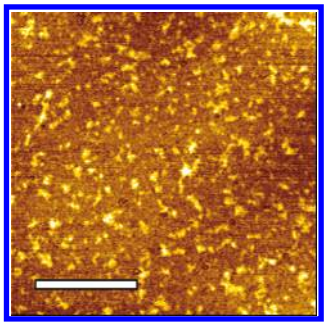

Figure 4. AFM topographic image of the globules formed by casting a 20 μg/mL bare DNA solution (pH 5.2) on mica. The scale bar represents 1 μm.

therefore, DNA adsorbed on the lipid membranes in the hydrate state forms 2-D arrays regulated by the water layers between DNA chains. When most water is removed by drying, the DNA chains may aggregate to remove the interstitial voids originally occupied by water; in this case, the ordered array structure may be destroyed. For the present DNA/dendrimer complex, however, the 2-D-ordered arrays are effectively preserved after drying. Here, the positively charged dendrimer plays the role of bridging DNA to the mica surface by an electrostatic force, which stabilizes the ordered arrays in the dry state.

To verify that bridging by the dendrimer is necessary to construct the ordered arrays, we used drop-casting to deposit a 20 μg/mL bare DNA solution on mica under the same conditions as those adopted for the complex solution. As can be seen from the AFM micrograph in Figure 4, bare DNA chains do not form ordered arrays in the dry state; instead, they collapse into globules to reduce their contact with the negatively charged mica.

Conclusions

We have presented a simple approach for constructing 2-D-ordered DNA arrays through the adsorption of a positively overcharged DNA/PAMAM G2 complex on a mica surface. This adsorption process was driven by the electrostatic attraction between the complex and the slightly negatively charged mica surface. As more and more rodlike complexes condense onto the substrate, they have to pack into ordered arrays to increase the surface density of the adsorbed complexes and hence the attractive free energy of adsorption. The use of electrostatic interactions in this context may potentially lead to new possibilities in supramolecular self-assembly and nanostructure engineering associated with biomolecules.

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Supporting Information Available: Additional AFM topographic images showing the formation of 2-D-ordered DNA arrays on a mica surface at different places in the same sample as that used to obtain the image in Figure 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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