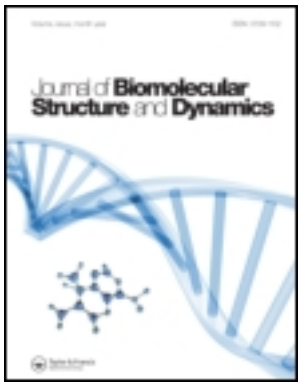

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Branched Nanowire Based Guanine Rich Oligonucleotides

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Abstract

Self-assembly and aggregation of guanine rich sequences can provide useful insights into DNA nanotechnology and telomeric structure and function. In this paper, we designed a guanine rich sequence d(GGCGTTTTGCGG). We found that it can form stable structure in appropriate condition and it exhibits an anomalous CD spectra. This structures can be imaged in ambient environment with a Nanoscope III AFM (Digital Instruments). We found it forms branch structure and long multistrand DNA nanowire after incubation at 37°C for 6-12 hours in 25 mM TE (pH=8.0) + 5 mM Mg^{2+} + 50 mM K^{+}. The ability to self-assemble into branches and long wires not only clearly demonstrate its potential as scaffold structures for nanotechnology, but also give aids to understand telomeric structure further. We have proposed a model to explain how these structures formed.

Introduction

It is well known that nucleic acids are polymorphic (1). DNA can adapt a number of conformations. Scientists have built a large number of potentially useful rigid nanostructures including molecular scale rods, rings, springs, cubes, spheres, tetrahedrons, hollow tubes, propellors, tongs, and wire-frame nanostructures of many shapes made of DNA (2,3). They have begun experimenting with these molecular construction kits. A DNA actuator made by Seeman et al (4) has two double crossover ends and a special sequence that can transit from the regular B-form to the unusual Z-form. Moreover, Seeman et al (5) has succeeded in making 2D regular carpets by letting two types of double crossover building blocks with distinct sequence hybridize together. DNA will find widely application in nanotechnology, and the key to success is coming up with a rigid DNA motif. Years ago, three way and four way junctions were used for nanoarchitecture, but they produce a panoply of cyclic products in ligation-closure experiment, suggesting that they are floppy rather than stiff (6,7). The double crossover DNA molecules are more rigid than duplex DNA as nanopart for nanotechnology. It has been shown that guanine rich (G-rich) oligomers can form intermolecular or intramolecular quadruplexs, depending on the chain length and intervening non-guanine nucleotides, with strong dependence on the monovalent cations such as K^{+} and Na^{+} (8). Specially, the sequences with several consecutive terminal guanine residues can form stable high-molecular-weight aggregation include frayed wires and quadruplexes according to the sequence and environmental effects (9-12). G-wire is consisted of a series of quadruplexes and is possibly a useful nanopart for nanoarchitecture, which has greater rigidity than duplex DNA. For example, a G-wire was self-assembled from d(GGGGTTGGGG) (13,14). The quadruplex based on d(GGGGTTGGGG) comprise of four G•G•G•G tetrads, and a new quadruplex with two G•G•G•G and two G•C•G•C was found (15,16). The two guanine rich sequences

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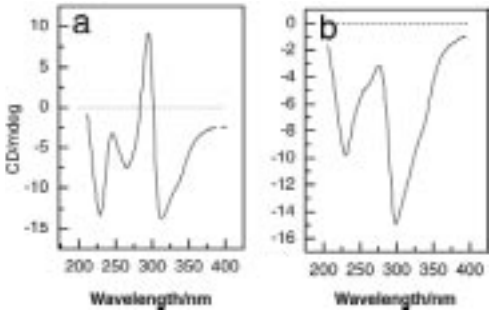

Figure. 1: CD spectra after self-assembly in TE (pH=8.0) + 5 mM Mg^{2+} + 50mM K^{+} (a) d(GGGGTTTTGGGG) (b) d(GGCGTTTTGCGG)

(d(GGGGTTTTGGGG) and d(GGCGTTTTGCGG)) were synthesized which can form the two quadruplexes described before respectively. We use Circular Dichroism (CD) and Atomic Force Microscopy (AFM) to study the structural properties of the noncovalent complexes of the two sequences.

Materials and Methods

Oligonucleotides. Synthetic oligonucleotides (d(GGGGTTTTGGGG) and d(GGCGTTTTGCGG)) were purchased from CyberSyn, BJ., and used without further purification. These oligomers were purified by the vender via reverse phase oligonucleotide purification cartridges and exhibited single-band electrophoretic mobilities in denaturing polyacrylamide gel electrophoresis. Concentrations of oligonucleotides were determined by measuring absorbances at 260 nm. (Possibly noncovalent complexes being formed, adsorbances were measured after denatured.)

Formation of Noncovalent Complexes. The oligonucleotide was dissolved into 25 mM Tris-HCl (pH 8.0), 5 mM Mg^{2+}, 50 mM K^{+}. Reaction was kept for 6-12 hours at 37°C then stopped by cooling the solution to 0°C.

Circular Dichroic spectra. CD spectra were made by a Jasco J-720 spectropolarimeter. Samples were measured at room temperature with use of special cells. (All measurement is done at room temperature unless specified.)

Atomic Force Microscopy Imaging. A drop of sample (about 2 μL) was spotted on freshly cleaved mica and left to stabilize about 2 min. Before washing the salt by super pure water (Millipore) the sample was dried by lighting or compressed N_2. Imaging was performed in tapping mode with a Nanoscope III (Digital Instruments), using a 125 μm Si tapping tip.

Results and Discussion

CD spectra

CD spectra can inspect the formation of G-quadruplex. There are two basic kinds of CD spectra usually observed for telomeric DNAs (17), one form exhibits a positive CD band at ~265 nm and a negative band at ~240 nm (type I), while the other form exhibits a positive band at ~295 nm and a negative band at ~260 nm (type II). These two CD spectra appear to be strongly correlated to the conformation of the G-quartet core. The parallel quadruplex structure (linear conformation) exhibits as type I, and the antiparallel quadruplex (folded conformation) does as type II. The CD spectra of noncovalent complex of d(GGGGTTTTGGGG) were shown in Fig. 1a. It exhibits a type II CD spectrum as judged by a characteristic signature with a 295 nm positive and 265 nm negative bands. The d(GGGGTTTTGGGG) adopt a G-quartet structured formed by hairpin dimerization (antiparallel quadruplex). As to d(GGCGTTTTGCGG), it's CD spectra in Fig. 1b exhibits a CD spectrum of neither type I nor type II with a 275 nm negative peak, a 298 nm and a 230 nm negative valleys. It is negative from 200 to 400 nm, and sometimes the ψ (PSI) type looks like this profile. But a ψ type was often been induced by higher concentration of polymer or salt, and DNA becomes a compact conformation with a tertiary structure. In this experiment, no else polymer exists except DNA, and cations in buffer are Mg^{2+} and K^{+} with concentration only 5 mM and 50 mM respectively. ψ type CD spectra is also a characteristic of superamolecular self-assembly aggregation of $d(XGG)_4$ under proper conditions (18-20). Specially, Mg^{2+} can facilitates the large ψ type CD spectra. And somewhat the superamolecular chirality possibly induces this result. We suggest that there is perhaps a new structure produced which is different from folded or linear conformation, and possibly be multistrand superamolecular DNA (such as G8-wire).

CD spectra suggest a new structure possibly formed. We can use AFM to image it. AFM can provide some useful structure information include height, width, etc. In this experiment, we use pBR322 DNA (a duplex DNA) as a reference to study the structure of the two sequence's noncovalent complexes.

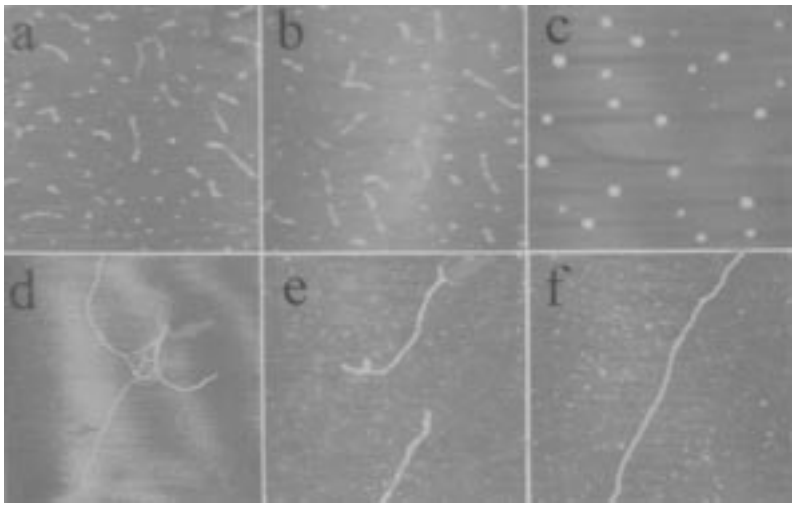

Figure 2: Tapping mode AFM images. (a) and (b) pBR322 plasmid DNA . (c) d(GGGGTTTTGGGG) after self-assembly in TE (pH=8.0) + 5 mM Mg^{2+} + 50mM K^{+}. (d)-(f) d(GGCGTTTTGCGG) after self-assembly in TE (pH=8.0) + 5 mM Mg^{2+} + 50mM K^{+}. (Scan size: (a) 4500x4500 (b) 4000x4000 (c) 3884x3884 (d) 6460x6460 (e) 3000x3000 (f) 1725x1725 nm^2.

The measured height from AFM images (Fig. 2a-b) of the double strand pBR322 plasmid DNA is about 0.55 ± 0.22 nm high, and the histogram of height distribution was shown in Fig. 3a. (The pBR322 plasmid DNA was dissolved in the same buffer as the two G-rich sequences.)

Apparently d(GGGGTTTTGGGG) aggregates into a kind of spheres (Fig. 2c) with about 41.67 nm diameter and 1.65 nm high. According to the CD spectra, the folded structure was self-assembled first, and then aggregated into these spheres with facility of 5 mM Mg^{2+}.

As to d(GGCGTTTTGCGG), AFM images are shown in Fig. 2d-f. First, one can find that d(GGCGTTTTGCGG) can be self-assembled into nanowires. Second, there are branches in Fig. 2d-e, and some branches can make up a web structure.

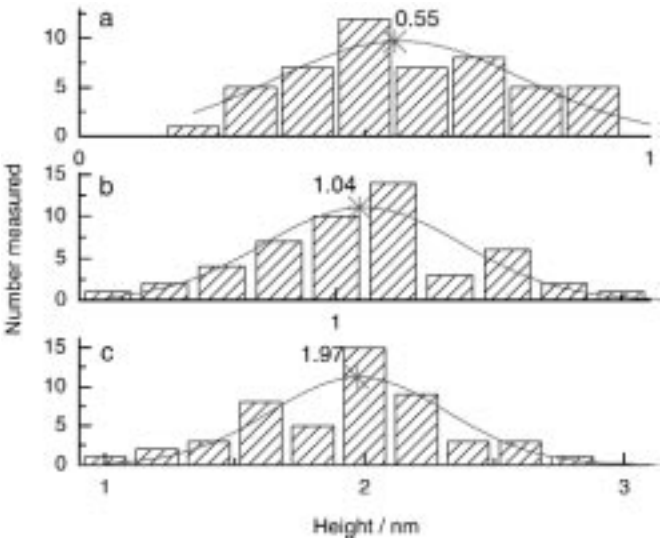

Figure 3: Statistics of the AFM measurements of the nanowires seen in Fig. 2. The histogram of the distribution of (a) plasmid DNA's height. (b) the nanowire in Fig. 2f. (c) the thickest nanowire in Fig. 2d.

Lastly, wires found in Fig. 2d have different apparent height. The histogram graphs of the height of these DNA nanowires were shown in Fig. 3. The wire in Fig. 2f is longer than 2000 nm, 1.04 ± 0.17 nm high, and the thickest wire in Fig. 2d (see

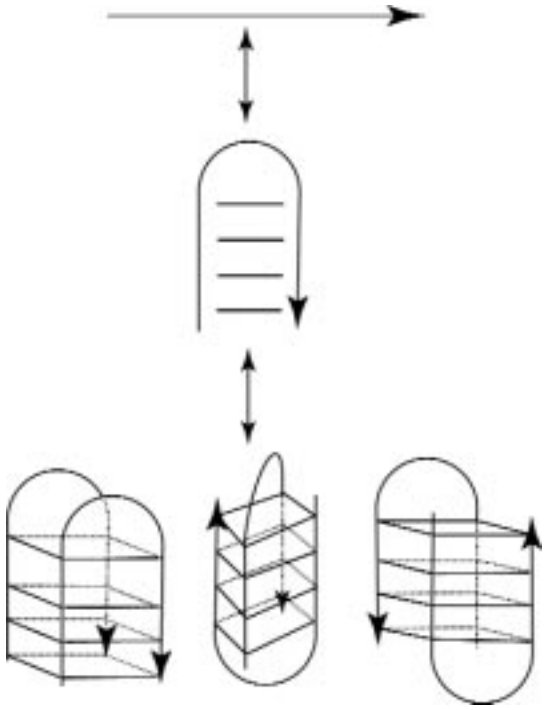

Figure 4: The self-assembly pathway of d(GGGGTTTTGGGG).

upright arrow) is 1.97 ± 0.34 nm high. These suggest that several kinds of multistrand DNA were formed. The height of G-wires depends upon the growth conditions, and was found to be two to three times greater than the height of plasmid DNA (14). The height of the nanowire in Fig. 2f is about twice high as plasmid DNA. Vesenka et al (13,14) have measured G-wire's height, and the height of those G-wires is 2.0 ± 0.5 nm. But no direct evidence proves those wires are four strand wires. Now, in our results, we found a kind of wire 1.05 nm high, which is lower than 2.0 nm, but much higher than the plasmid DNA. It is possible a four strand wire, and the higher wires are more complex multistrand wires. We can clearly see that three wires intersect a single junction point (see upright arrow in Fig. 2d and arrow in Fig. 2e), and three wires are of dissimilar height and width. It is seemed that two sub-branches deviate flow from a mainstream. The web or branch structure and multistrand model can give a reasonable explanation to the CD spectra's anomalous negative (18). The thick wire possibly behaves superamolecular chirality which can induces ψ type CD spectra. Otherwise CD spectra can sometimes change drastically when the molecule becomes larger. When the molecule's size reaches to the wavelength of the light used in the CD experiment, anomalous spectral changes occur. The web or branch structure and multistrand DNA is possibly the origin of the anomalous CD spectra.

Alternative pathways of structure formation of guanine rich oligonucleotides have been proposed (14). It can not account for the branch structure and multistrand. According to this model, we propose other alternative pathways too. The oligonucleotide d(GGGGTTTTGGGG) is assembled as Fig. 4 as a folded structure. In fact, there is an equilibrium between folded structure and linear structure. But the equilibrium is reached over a long period of time due to the extremely slow kinetics of formation / dissociation which typical for four-stranded quadruplexs. The CD spectra show as type I, so we think that d(GGGGTTTTGGGG) was assembled to folded structure as discussed previously. Even if linear structure formed, which was of trace, so the pathway to linear structure was not draw in Fig. 4. The d(GGCGTTTTGCGG) is assembled as Fig. 5. The quadruplex consist of multilayer arrangement of G•G•G•G and G•C•G•C tetrads. The G•C•G•C tetrads have been reported (15,16), this tetrad composed of the unusual combination of two guanine and two cytosine bases, all in the anti conformation, was sandwiched by standard guanine quartets. The G•C•G•C tetrad maintains it's G-C Watson-Crick hydrogen

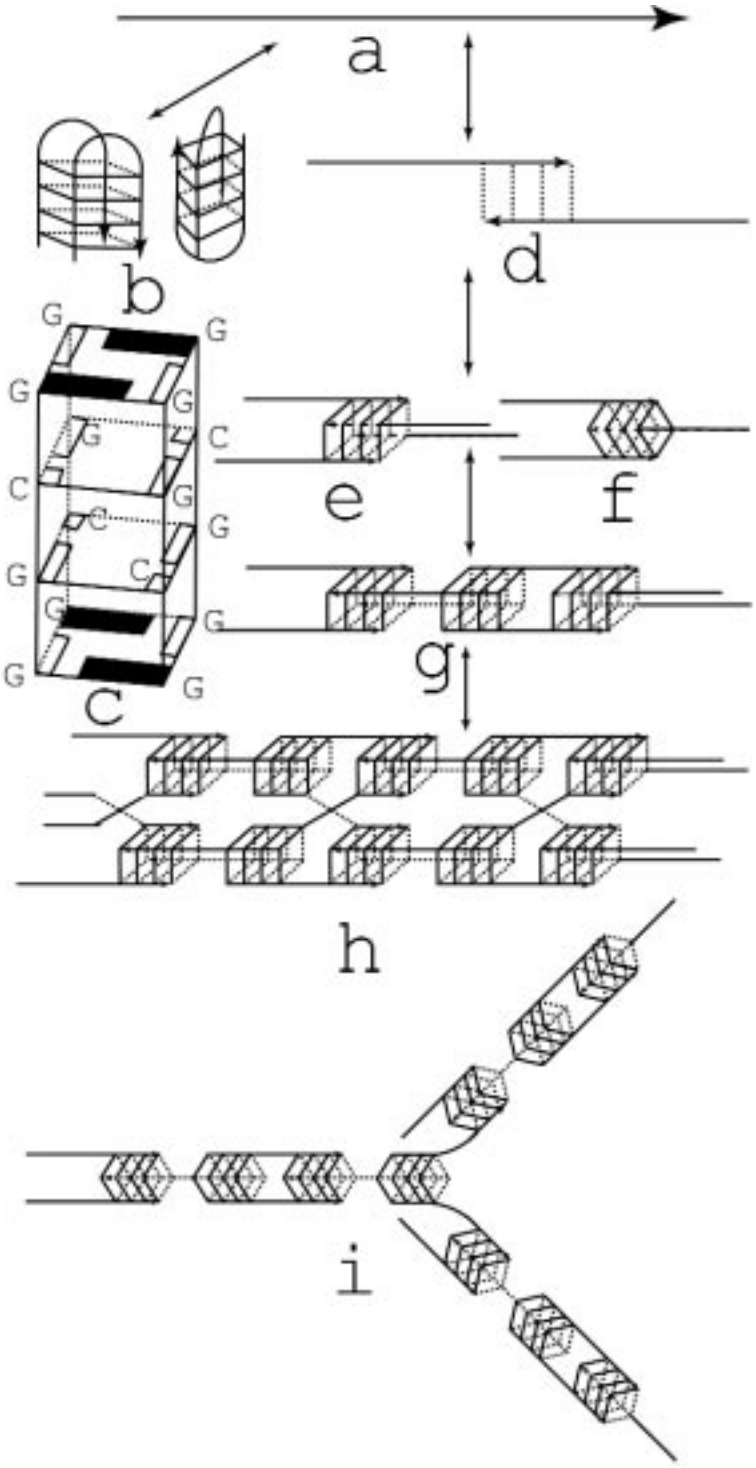

Figure. 5: The pathways of d(GGCGTTTTGCGG)'s self-assembly. (a) d(GGCGTTTTGCGG). (b) Possible hair-pin folded dimer structure. (c) G-quadruplex with two G•C•G•C tetrads sandwiched by two G•G•G•G. (d) a dimer consisted of two d(GGCGTTTTGCGG). (e) G4-wire scaffold consisted of four d(GGCGTTTTGCGG). (f) The diagonal section of (d). (g) G4-wire. (h) The model of G8-wire. (i) The model of G-wire branch

bonding and interacts with the opposing C-G by major groove interactions. In general, if n > 2, the linker Tn can form a loop. For example, d(GCGGTTTGCGG) can form a dimeric hairpin quadruplex (13,14). We assume that the T4 can form a loop in d(GGCGTTTTGCGG) as in Fig. 5, so the branched structure can form. Multiple branches can lead to a web structure. We assume that one strand of the G4-wire quadruplex is cross to another G4-wire and vice versa, this structure is greatly similar to the parallel Holliday junction if we regard other three strands of quadruplex as the side strand of the Holliday junction, then a G8-wire formed (Fig. 5h). Similarly more complex multistrand G-wire could form. Telomeric DNA is characterized by tandem repeats of guanine rich sequences, the branch or web structure and multistrand DNA perhaps has biological implications for telomeric structure and function.

Conclusion

By using CD and AFM, we found a special guanine rich sequence d(GGCGTTTTGCGG) can form branch structure and multistrand DNA. The ability to self-assemble into branches and long wires not only clearly demonstrate its potential as scaffold structures for nanotechnology, but also give aids to understand telomeric structure further. We have proposed a model to explain how these structures formed.

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