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Artificial, Parallel, Left-Handed DNA Helices

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Supporting Information

ABSTRACT: This communication reports an engineered DNA architecture. It contains multiple domains of half-turn-long, standard B-DNA duplexes. While each helical domain is right-handed and its two component strands are antiparallel, the global architecture is left-handed and the two component DNA strands are oriented parallel to each other.

In the common B-form duplex, two parallel, complementary DNA strands intertwine with each other in a right-handed fashion.[1−6] DNA can also exist in the A form, a slight variation of the B-form structure.[3,4,6,7] A more dramatically different structure is Z-form DNA where the duplex is left-handed.[8] In all of these well-known conformations, the two component strands are antiparallel to each other. Here we report an unusual DNA architecture (a parallel, left-handed duplex) that is dramatically different from all of the above-mentioned conformations.

The parallel, left-handed DNA duplex contains two intertwined DNA strands that are structured into multiple domains (Figure 1). Each domain is a half-turn B-DNA duplex, where the two component strands are antiparallel in a right-handed sense and the base-pair planes are perpendicular to the short, local helical axes. However, those domains together exhibit a globally left-handed sense, and the two component DNA strands run in parallel along the global helix with the base-pair planes parallel to the long, global helix axis. In this architecture, many base pairs have exposed surfaces that are not involved in base stacking in the local helix. The exposed base pairs lining the two opposite sides of the helix form continuous aromatic planes, which would promote extensive base-stacking interactions among the left-handed duplexes and cause them to form large aggregates. Such aggregation can be prevented by the introduction of extra, unpaired bases [e.g., thymines (T's)] at both ends of the DNA duplex. These extra bases produce steric hindrance that interferes with the interhelix base stacking. In addition, the extra bases can also stack onto the nearby exposed base pairs and stabilize the left-handed duplexes.

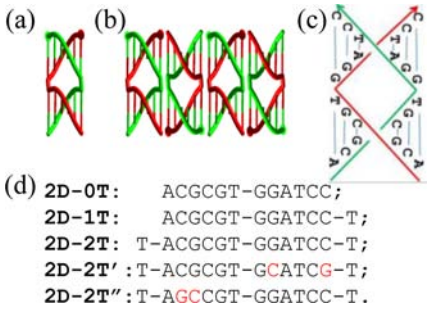

Figure 1. Parallel, left-handed DNA duplexes with two half-turn domains. (a) Structural model (drawn with Nanoengineer, NanoRex Inc.). (b) Base stacking among the left-handed DNA duplexes leads to large aggregates (shown as an example). (c) Composition of one left-handed DNA duplex studied here. It should be noted that the two component strands have identical sequences. (d) DNA sequences of the derived molecules. Mutated bases (red) introduce mismatches. In the names of the DNA strands, "2D" stands for two domains and "*n*T" indicates that the total number of extra T's added is *n*.

We first investigated the formation of the left-handed DNA helix by native polyacrylamide gel electrophoresis (PAGE) in the presence of Mg^{2+}. The experimental data were consistent with expectations (Figure 2). At low temperature (e.g., 5 °C), **2D-0T**, **-1T**, and **-2T** all assembled into complexes containing more than one strand. **2D-2T** was 14 bases long and had one extra free T at each end of the strand. The resulting complex

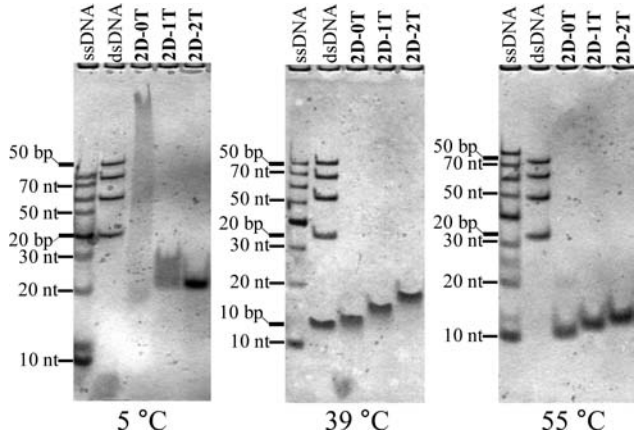

Figure 2. Native polyacrylamide gel electrophoresis (PAGE) analysis of the two-domain, left-handed DNA structures in the presence of Mg^{2+} at different temperatures. The identities of the DNA samples are indicated above the gel images. For each gel, the left two lanes are size markers of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). The marker sizes [by nucleotides (nt) or base pairs (bp)] are indicated to the left of each gel image. The three gels contained the same size markers, except that the gel at 39 °C had one extra band corresponding to 10 bp in the dsDNA marker lane. The mobility change of the DNA molecules relative to the size markers should be noted.

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showed a sharp band in the gel. Its electrophoretic mobility was consistent with the mobility of a **2D-2T** dimer, indicating that the individual **2D-2T** dimer was stable under these conditions. **2D-1T** was 13 bases long and had one extra T at the 3′ end. The DNA sample appeared as a narrowly distributed smear whose mobility was slightly lower than that of the dimer, indicating that the **2D-1T** dimers were base-stacked onto each other to form larger complexes. However, such base stacking was not very strong under the experimental conditions, and the large complexes continuously dissociated during electrophoresis, resulting in a smear in the gel instead of a sharp band. **2D-0T** was 12 bases long and had no extra free T at either end of the DNA strand. In the gel, it appeared as a smear with wide distribution. The mobility ranged from that of **2D-0T** dimers to those of very large complexes (far larger than a complex containing 10 copies of **2D-0T**), indicating strong interhelix base stacking. At a higher temperature (39 °C), the interhelix base stacking became weaker, and all three molecules formed individual dimers, appearing as sharp bands with the expected mobilities. A further increase in the experimental temperature (to 55 °C) denatured the DNA dimers, resulting in only single strands.

The PAGE data suggested that the DNA molecules assembled into dimers. The **2D-2T** dimers were stable and dispersed in the solution as individual complexes. However, both the **2D-0T** and **2D-1T** dimers further assembled into large aggregates through base-stacking interactions. The extra, free T's at the ends of the left-handed duplexes prevented the interhelix base stacking and promoted the existence of individual dimers instead of large aggregates. In a solution with lower ionic strength (in the absence of Mg^{2+}), the overall trend was the same, but the corresponding temperatures were lower (Figure S1 in the Supporting Information).

The DNA assembly behavior was confirmed by thermal denaturation analysis (Figure 3a), in which the DNA

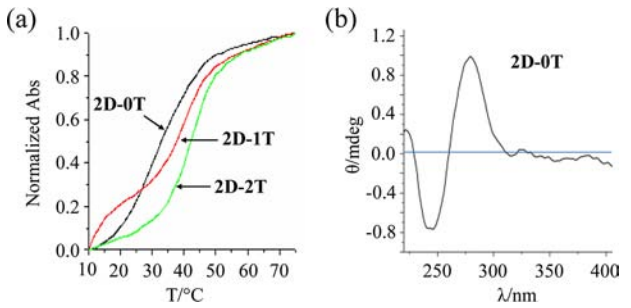

Figure 3. Spectroscopic analysis of the two-domain, left-handed DNA duplexes: (a) thermal denaturation as monitored by UV absorption at 260 nm (4 μM DNA); (b) CD spectrum of **2D-0T**.

absorption at 260 nm was monitored while the solution temperature was increased. **2D-2T** showed a single transition at 42 °C, corresponding to dimer melting. **2D-1T** showed two distinct transitions: a large one for the dimer melting at high temperature (40 °C) and a small one at lower temperature (12 °C) corresponding to loss of the interhelix base stacking. This is consistent with the results of the PAGE analysis showing that the interhelix base stacking for **2D-1T** existed but was weak. For **2D-0T**, the two transitions (centered at 31 and 45 °C, respectively) significantly overlapped with each other. The base stacking for **2D-0T** was strong and had a higher melting temperature (T_m) than that of **2D-1T** did.

The interduplex base stacking was also influenced by the DNA concentration. Lowering the DNA concentration weakened the interduplex base stacking (Figure S2). For example, at a DNA concentration of 1 μM, **2D-1T** had only one transition (corresponding to melting of the left-handed duplex). Similarly, the aggregates of **2D-0T** duplexes became less stable, and the second transition (for interduplex base stacking) occurred at a much lower temperature (18 °C).

To probe the local DNA conformation, we checked the circular dichroism (CD) spectra of the DNA samples. The CD spectra are sensitive to DNA secondary structures and are commonly used to identify the DNA conformation. Our experimental data (Figure 3b and Figure S3) confirmed that all of the DNA samples had local structures of typical B-conformation, consistent with expectations.[9]

Both domains in the left-handed DNA duplex formed and contributed to the overall structural stability. Disruption of any one domain decreased the stability of the DNA complex. In a control experiment, we prepared two DNA mutants (**2D-2T′** and **2D-2T″**), each containing two mutations in one domain (Figure 1d). Each mutant DNA can form only one half-turn domain, and the other half would remain unpaired because of the lack of sequence complementarity. The mutant DNA strands could not form stable DNA complexes as **2D-2T** did (Figure 4). For example, **2D-2T** formed stable left-handed duplexes (dimers) at 39 °C, while the mutant DNAs could not form dimers but existed as monomers.

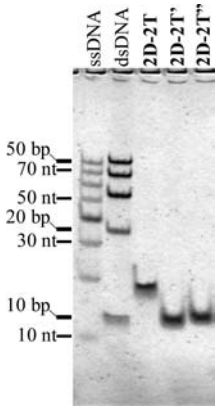

Figure 4. Native PAGE (at 39 °C) showed that both domains contributed to the stability of the left-handed DNA duplex.

The left-handed, parallel architecture is a general structure and not limited to a length of two domains. We investigated such architectures with three (Figures S4–S7) and four half-turn domains (Figures S8–S11). They both formed left-handed, parallel duplexes having a strong tendency to associate into large complexes through extensive base-stacking interactions among the left-handed duplexes. The inclusion of extra, free T's at the ends disrupted the base stacking and promoted the existence of the individual duplexes.

In summary, we have reported an unusual DNA architecture: a globally left-handed, parallel DNA duplex containing multiple domains of half-turn-long, regular right-handed, antiparallel B-DNA. A similar structure was proposed previously but dismissed.[10] The previous study attributed the observed large DNA complexes to DNA base pairing among multiple strands and led to the development of the paranemic crossover (PX) structure, an important nanomotif in DNA nanotechnology. In

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the current study, we suggest that the formation of large complexes is due to base stacking among left-handed DNA duplexes, and we have experimentally demonstrated this architecture. We expect that the reported DNA structure will find applications in structural DNA nanotechnology.[11,12] Such a structure would allow us to readily build DNA topological objects that contain left-handed crossings (such as in Borromean rings and figure-eight knots) and overcome the experimental difficulty imposed by the left-handed Z-DNA or synthetic challenges of modified DNA structures.[13,14] In addition, because such a structure can exist under physiological conditions (close to neutral pH and in the presence of divalent cations), it raises a question: do similar structures (particularly for RNA) exist in cells? Furthermore, this study might provide new insight to the prediction of DNA secondary structures. In the future, it might be worthwhile to take such architectures into account when considering nucleic acid interactions.

■ ASSOCIATED CONTENT

Supporting Information

Materials and Methods and additional experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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