Research Article

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The Helicity of a DNA-2'-Fluoro DNA Hybrid Duplex Structure

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Abstract

Structural DNA nanotechnology is a system whereby branched DNA molecules are fashioned into objects, or 1D, 2D and 3D lattices, as well as nanomechanical devices. Normally, one is dealing with the usual B-form DNA molecule, but variations on this theme can lead to alterations in both the structures and the properties of the constructs. 2'-Fluoro DNA (FDNA), wherein one of the hydrogen atoms of the 2' carbon is replaced by a fluorine atom, is a minimal steric perturbation on the structure of the DNA backbone. The helical structure of this duplex is of great interest for applications in structural DNA nanotechnology, because the DNA-FDNA hybrid assumes an A-form double helix, without the instabilities associated with RNA. Here we have used an atomic force microscopic method to estimate the helicity of DNA-FDNA hybrids, and we find that the structure contains 11.8 nucleotide pairs per helical turn with an error of ± 0.6 nucleotide pairs, similar to other A-form molecules.

Keywords: DNA Helicity, Modified DNA Backbones, DNA Nanotechnology, Atomic Force Microscopy, 2D DNA Arrays.

Introduction

Nucleic acid-based nanotechnology entails the use of branched oligonucleotides (e.g. DNA, RNA, PNA or LNA molecules) to design and fabricate constructs of structural and topological importance, as well as to construct nanoscale objects, lattices and devices [1,2]. DNA is a suitable candidate for this purpose because of its high level of programmability, reliable base-pairing specificity, high physicochemical stability, and readily automated synthesis [3,4]. DNA can assume several helical forms, most commonly the A-form and the B-form, and knowing the helical properties of a particular variant of DNA is a step towards the introduction of non-natural nucleic acids to build more varied structures.

2'-Fluoro DNA (FDNA) is a nucleic acid variant that assumes only the A-form double helix (**Fig. 1**) when paired with RNA, and is expected to have dominant helicity when hybridized with standard DNA. The introduction of the fluoro group is a minimal stereo chemical perturbation of the DNA backbone. Apart from its nuclease resistance, it shows a strong pairing affinity i.e. a higher melting transition than ordinary DNA and RNA duplexes [5,6]. These properties of FDNA prompted us to check the suitability of constructing ordered DNA arrays with these molecules and to estimate the helical repeats therein. The method we use is based on the formation of 2D DNA arrays, as reported earlier by Lukeman, et al. [7,8].

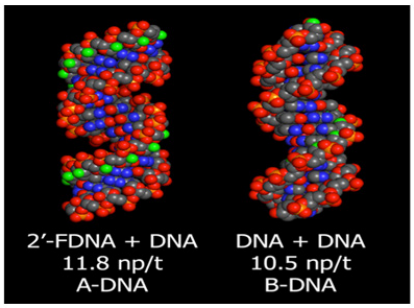

Figure 1: The A-Form of DNA (left) and the B-Form of DNA (right). The detailed structures are noted for the species used here.

The substitution of a DNA-FDNA hybrid into the array will perturb the formation of the array, since the helicity may differ from that of the basic DNA unit. This method uses a two-tile system to establish the helicity of an unknown modified nucleotide component (**Fig. 2**).

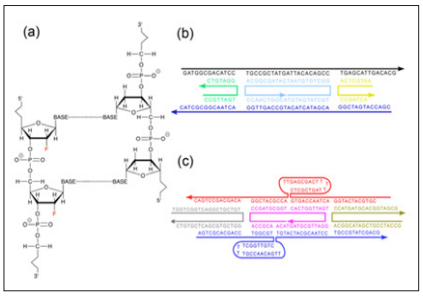

Figure 2: The chemical structure of the DNA-FDNA duplex and the DX motifs used in this study. (a) shows the DNA-FDNA

dinucleotide pair. The tile in (b) is the **A** tile: the yellow strand and the green strand consist of FDNA. (c) contains the **B*** tile consisting only of conventional DNA.

Each of the double crossover (DX) tile pairs is designed to repeat roughly every 32 nm (for conventional DNA), leading to an exact repeat, so the 2D array can form readily [9]. One tile, the A tile, contains two copies of the DNA-XNA (DNA-FDNA in this case), and the size of the other tile is adjusted to yield a planar array that can be visualized in an atomic force microscope (**Fig. 3**).

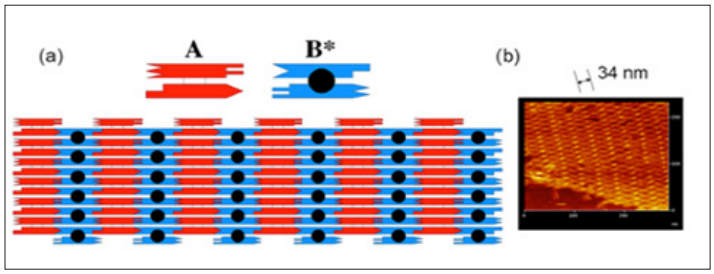

Figure 3: The 2D DX Array. Panel (a) shows multiple tiles forming a two-dimensional array, with complementary sticky ends represented as geometrical shapes. Hairpins are represented as black circles. Panel (b) is an atomic force micrograph with a stripe spacing of 34 nm.

The adjustable tile, the B* tile, also contains a domain that is directed away from the plane of the 2D array, yielding a striped pattern when the 2D array forms. The presence of the striped pattern is a good indication that the array has formed correctly. The number of nucleotides in the adjustable tile is varied to establish the helicity of the DNA-FDNA double helix. The resulting 2D arrays are visualized using atomic force microscopy (AFM), and the helical repeat of a DNA-FDNA hybrid duplex is derived to be 12.1 nucleotide pairs per turn (np/t).

Materials and Methods

Two versions of the A tile were assembled: The **AD** tile which is entirely DNA, and the **AF** tile with two short crossover strands (green and yellow in Fig. 2b) containing exclusively 2'-FDNA. Simultaneously, twelve versions of **B*** tiles were assembled, $\mathbf{B_0}$-$\mathbf{B_{11}}$. The subscript indicates the number of nucleotide pairs added to compensate for the possible twist difference when **AF** is incorporated into the arrays.

Design, Synthesis and Purification of DNA Strands

The two FDNA strands, 15 nucleotides in length used in the **A** tile, were synthesized by an Automated DNA Synthesizer (*Applied Biosystems 394*) via routine phosphoramidite chemistry [4]. All other DNA strands were purchased from Integrated DNA Technologies (Coralville, IA) (www.idtdna.com).

DNA strands were dissolved in denaturing dye buffer (0.1% Xylene Cyanol FF tracking dye in 90% formamide with 1 mM EDTA, 10 mM NaOH) and purified by 10-20% denaturing PAGE (depending on strand length) containing 8.3 M urea in TBE buffer (89 mMTris-HCl, pH 8.0), 89 mM boric acid, 2 mM EDTA). The desired bands were cut from the gel, eluted and precipitated by ethanol. The concentration of each strand was measured by absorbance at 260 nm in double distilled water.

Annealing of Tiles and Formation of 2D Lattices

For the formation of the desired 2D lattices, the strands of both **A** and **B*** tiles were mixed together in a stoichiometric ratio in 1x TAE/Mg buffer (40 mM Tris, pH 7.8, 2 mM EDTA, 12.5 mM Mg/Acetate). The final DNA concentration of the sample was 1 µM. The mixture was then annealed slowly from 90 ˚C to room temperature for a period of about 48 hours in a 2L insulated water bath.

Preparation of 2D Crystals and AFM Imaging

2-3 µL of the annealed DNA lattices were spotted on a freshly cleaved mica surface (Ted Pella, Inc.) and allowed to adsorb onto the surface for 2 min. An additional 25 µL of fresh buffer was added to both the mica and the fluid cell. Imaging was performed under 1x TAE/Mg in tapping mode AFM on a Nano scope IV (Digital Instruments) using commercial cantilevers with Silicon Nitride (Si_3N_4) tips.

Results

We used the AFM to monitor the formation of arrays that included all combinations of the tiles, as shown in Fig. 4. The **AD** tile, consisting of DNA only, forms arrays cleanly with tiles $\mathbf{B_0}$, $\mathbf{B_1}$, and to some extent with $\mathbf{B_2}$, as expected. As the number of added bases increases, the self-assembly fails and random linear aggregates or short linear arrays are obtained. Non-denaturing gels showing the formation of all tiles used are shown in Supplementary Figures 1-4. Other AFM data are shown in Supplementary Figure 5. The sequences of all tiles are also shown in the Supplementary Information.

By contrast, the corresponding array combining the **AF** tile with $\mathbf{B_0}$ is formed very poorly. However, combining the **AF** tile with $\mathbf{B_1}$, $\mathbf{B_2}$ and (to some extent) $\mathbf{B_3}$ leads to well-patterned arrays; these arrays display stripes with the expected 34 nm periodicity. The remaining combinations do not form 2D arrays, but rather random aggregates similar to the **AD**-$\mathbf{B_2}$ to **AD**-$\mathbf{B_{11}}$. These data demonstrate the incorporation of 2'-FDNA oligomers into a nucleic acid nanostructure capable of forming ordered 2D arrays.

The robustness of the resulting two-dimensional array was observed to be highest at **AF**=$\mathbf{B_2}$ for the hybrid and decreased in structural integrity with each successive image, eventually failing to form any 2D array whatsoever. As expected, the helicity of standard DNA peaked at **AD**-$\mathbf{B_0}$, corresponding to a helical repeat of 10.5 np/t, which agrees with previous estimates.

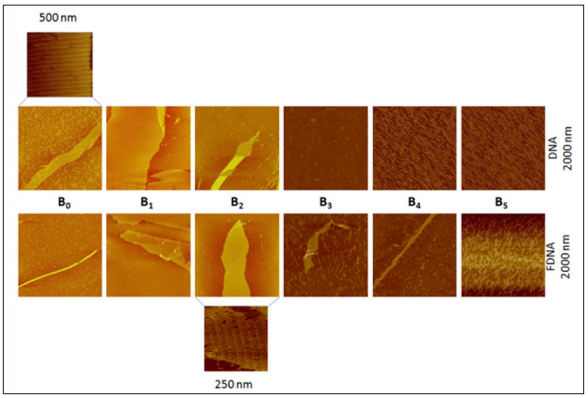

Figure 4: AFM images of arrays formed from the tiles **AD** and **AF** with tiles $\mathbf{B_0}$-$\mathbf{B_5}$. The images in the two columns are a 200 nm field with a height scale of 6.0 nm. **AD**-$\mathbf{B_0}$ and **AF**=$\mathbf{B_2}$ have zoomed images (scan scale 500 nm and 250 nm) on the top and bottom, respectively, showing the periodic striped patterns of the array.

For the formation of extended 2D arrays, each arm of the **AF-B$_2$** tiles connected by sticky-ended cohesion must be 2.5 helical turns. The helical repeat of the DNA-FDNA segment of **AF** can be calculated assuming that the average helical repeat for the rest of the system remains at 10.5 np/t. The lengths of the four arms in the **AF-B$_2$** system correspond to an average helical repeat of 11.8np/t for the DNA-FDNA hybrid duplexes. This result is measured over the 15 DNA-FDNA base pairs present per tile.

Discussion

We have established the helicity of the DNA-FDNA hybrid to be about 11.8 nucleotide pairs per turn. Kawasaki et al [5]. established that the CD spectrum of DNA-FDNA is intermediate between A and B-form. This is a key result, because the presence of the fluorine atom on the C2' position is about as minimal a stereo-chemical perturbation as one could imagine in the absence of using an isotope of hydrogen. Thus, even though the perturbation is small, we have made what appears to be a helix with helical parameters closer to A-form than B-form. It should be possible to build FDNA-containing nanostructures with wider and shorter helices. For example, the tensegrity triangle containing FDNA in one of its helical strands is likely to have significantly different structural properties, and to produce significantly different crystals from the tensegrity triangle containing only conventional DNA [10,11].

Acknowledgments

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References

1. Seeman NC (1982) J Theor Biol 99: 237-247.
2. Seeman NC (2003) Nature 421: 427-431.
3. Seeman NC (2010) Ann Rev Biochem 79: 65-87.
4. Caruthers MH (1985) Science 230: 281-285.
5. Kawasaki AM, Casper MD, Freier SM, Lesnik EA, Zounes MC,et al. (1993) J Med Chem 36: 831-841.
6. Pallan P, Greene E, Jicman P, Pandey R, Manoharan M, et al. (2010) Nucl Acids Res 39: 3482-3945.
7. Winfree E, Liu F, Wenzler LA, Seeman NC (1998) Nature 394: 539-544.
8. Lukeman PS, Mittal AC, Seeman NC (2004) Chem Comm 15: 1694-1695.
9. Fu TJ, Seeman NC, (1993) Biochemistry 32: 3211-3220.
10. Liu D, Wang W, Deng Z, Walulu R, Mao C (2004) J Am Chem Soc 126: 2324-2325.
11. Zheng J, Birktoft JJ, Chen Y, Wang T, Sha R, et al. (2009) Nature 461: 74-77.