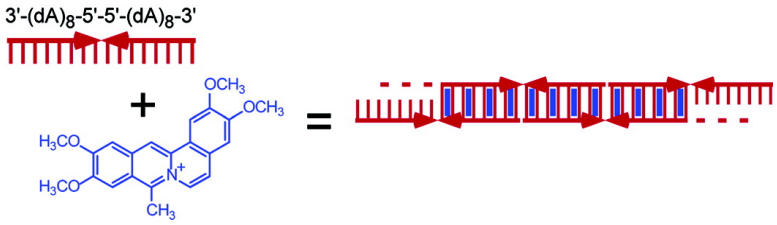
Communication

Assembly of an Antiparallel Homo-Adenine DNA Duplex by Small-Molecule Binding

zgl Persil, Catherine T. Santai, Swapan S. Jain, and Nicholas V. Hud

J. Am. Chem. Soc., **2004**, 126 (28), 8644-8645• DOI: 10.1021/ja0492891 • Publication Date (Web): 26 June 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/26/2004

Assembly of an Antiparallel Homo-Adenine DNA Duplex by Small-Molecule Binding

Özgül Persil, Catherine T. Santai, Swapan S. Jain, and Nicholas V. Hud*

School of Chemistry and Biochemistry, Parker H. Petit Institute of Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, Georgia 30332-0400

Received February 9, 2004; E-mail: hud@chemistry.gatech.edu

Molecules that bind DNA and trigger the formation of non-Watson−Crick secondary structures would be useful in the design of dynamic DNA nanostructures[1] and as potential leads for new therapeutic agents.[2] The small molecule coralyne (Figure 1a) was recently found to bind poly(dA) with a stoichiometry of one coralyne per four adenine bases, and to be released from poly(dA) in a cooperative melting transition around 50 °C.[3] These observations suggest that poly(dA) forms a coralyne-dependent secondary structure. However, the basic features of this putative homo-adenine structure, such as strand number (e.g., duplex vs quadruplex) and strand orientation (i.e., parallel vs antiparallel), are not obvious. Here we demonstrate that coralyne promotes the formation of an antiparallel homo-adenine duplex. Furthermore, the helix of this duplex is compatible with flanking Watson−Crick helices. To the best of our knowledge, this represents the first report of a homo-adenine duplex at neutral pH.

In Figure 1b we show an image of a nondenaturing polyacrylamide gel cast with 20 μM coralyne in which the oligonucleotides $(dA)_4$, $(dA)_8$, $(dA)_{16}$, and $(dA)_{32}$ were subjected to electrophoresis. Of these four oligonucleotides only $(dA)_4$, the shortest of the series, migrates with the mobility expected for a single-stranded oligonucleotide. Oligonucleotides $(dA)_8$, $(dA)_{16}$, and $(dA)_{32}$ apparently form complexes with coralyne that are too large to migrate appreciably through the gel matrix.

CD spectra shown in Figure 1c demonstrate that $(dA)_{16}$ undergoes a change in secondary structure in the presence of coralyne. The positive CD bands observed between 310 and 350 nm indicate the binding of coralyne within the chiral environment of DNA. These CD bands are quite pronounced for coralyne in the presence of $(dA)_{16}$ and $(dA)_{32}$ at 5 °C (Figure 1d). However, a sample of $(dA)_8$ containing the same concentration of coralyne and DNA (in nucleotide base) exhibits a lower degree of coralyne binding, and coralyne CD bands are not at all detected with $(dA)_4$ (Figure 1d). The positive correlation between $(dA)_n$-induced coralyne CD bands and evidence of high-molecular weight assemblies from gel electrophoresis studies indicate the formation of $(dA)_n$−coralyne assemblies that increase in stability with increasing oligonucleotide length. These results are consistent with the formation of a multistranded coralyne-dependent $(dA)_n$ secondary structure. Furthermore, UV absorption studies confirm that the stability of the $(dA)_n$−coralyne structure depends on both DNA and coralyne concentration (Supporting Information).

To directly examine the size and morphology of $(dA)_n$−coralyne assemblies, $(dA)_n$ oligonucleotides in solution with coralyne were deposited on mica and imaged using an atomic force microscope (AFM). A representative image from the $(dA)_{16}$ preparation is shown in Figure 2a, which reveals polymers of micrometers in length but only 1.6 nm (±0.3 nm) in height. Similar polymers were observed for $(dA)_8$ and $(dA)_{32}$ in the presence of coralyne, but not for $(dA)_4$, or any of the $(dA)_n$ oligonucleotides in the absence of coralyne (data not shown). The length of these molecular assemblies suggests the staggered pairing of $(dA)_n$ oligonucleotides into quasi-infinite multistranded polymers that minimize exposed ends of unstacked bases and maximize binding sites for coralyne.

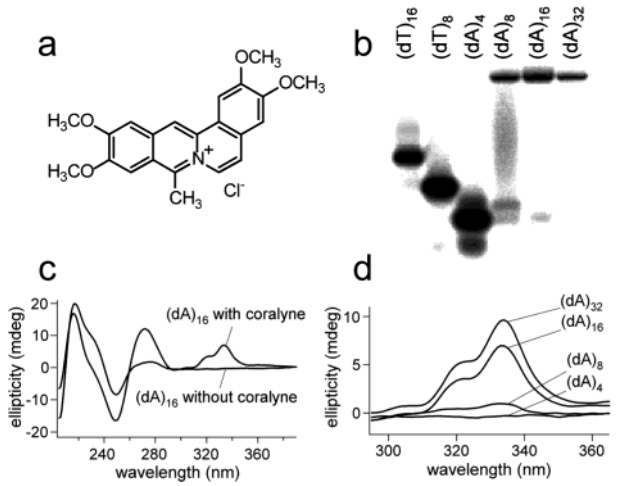


Figure 1. (a) Coralyne chloride. (b) Gel mobility experiment for $(dA)_n$ oligonucleotides in a nondenaturing 15% polyacrylamide gel cast with 20 μM coralyne (run at 4 °C ambient temperature). $(dT)_8$ and $(dT)_{16}$, which do not bind coralyne, serve as molecular weight markers. Samples loaded into the gel were 16 μM in nucleotide base and 16 μM in coralyne. (c) CD spectra of $(dA)_{16}$ (55 μM in nucleotide base) acquired at 5 °C in the absence and presence of 14 μM coralyne. (d) Selected region of CD spectra of four $(dA)_n$ oligonucleotides (each 55 μM in nucleotide base) with 14 μM coralyne at 5 °C.

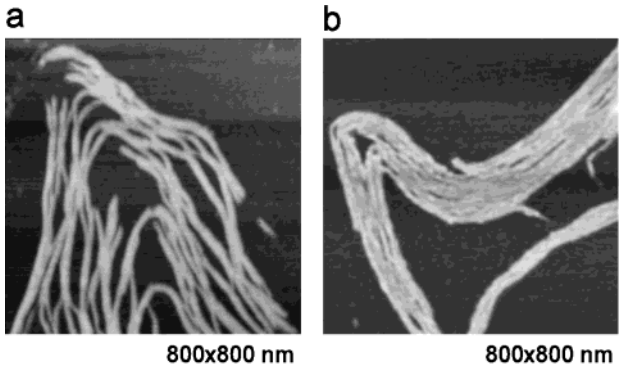


Figure 2. AFM images of homo-adenine−coralyne assemblies (a) $(dA)_{16}$ and (b) 3′-$(dA)_8$-5′-5′-$(dA)_8$-3′. Both samples were 220 μM in nucleotide base, 55 μM coralyne and imaged on freshly cleaved mica after drying. Polymers measure 1.6 nm (±0.3 nm) in height.

To investigate strand polarity in the $(dA)_n$−coralyne structures we prepared the oligonucleotide 3′-$(dA)_8$-5′-5′-$(dA)_8$-3′, an analogue of $(dA)_{16}$ that differs by a change in strand polarity at the center of the oligonucleotide. If the homo-adenine−coralyne secondary

10.1021/ja0492891 CCC: $27.50

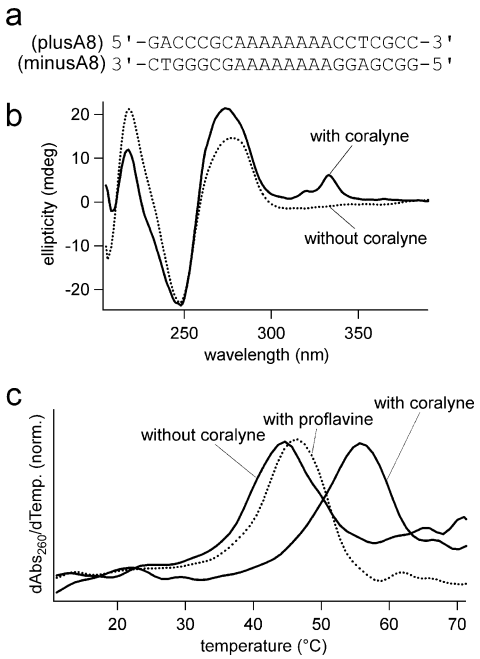
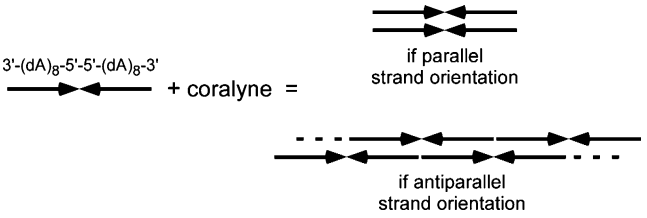


Figure 3. (a) Base alignment in the **plusA8·minusA8** duplex. (b) CD spectra of duplex **plusA8·minusA8** in the absence and presence of coralyne (acquired at 5 °C). (c) Derivative UV melting curves for duplex **plusA8·minusA8** in the absence and presence of coralyne and proflavine, respectively. All samples were 55 μM in adenine nucleotide. Samples with coralyne or proflavine were 14 μM in these molecules.

Scheme 1. Assembly Constraints Implicit to 3′-$(dA)_8$-5′-5′-$(dA)_8$-3′



structure contains only parallel DNA strands, then this particular oligonucleotide would be restricted to the assembly of structures only 16 nucleotides in length (Scheme 1). On the other hand, if the homo-adenine−coralyne structure contains antiparallel strands, then this oligonucleotide would assemble into quasi-infinite polymers (Scheme 1). The observation of micrometer-length fibers formed from 3′-$(dA)_8$-5′-5′-$(dA)_8$-3′ and coralyne indicates the inclusion of antiparallel strands in this structure (Figure 2b). A similar antiparallel strand arrangement is also likely to exist in the $(dA)_n$−coralyne assemblies; however, a parallel strand arrangement cannot presently be ruled out.

To determine if the $(dA)_n$−coralyne structure is composed of two strands, as opposed to three or more, we tested the ability for the homo-adenine−coralyne structure to be accommodated within a Watson−Crick duplex. This was accomplished by repeating the series of experiments described above with a duplex formed by the oligonucleotides 5′-GACCCGC-A_8-CCTCGCC-3′ (**plusA8**) and 5′-GGCGAGG-A_8-GCGGGTC-3′ (**minusA8**). These sequences were designed to form two 7-bp Watson−Crick duplexes that are separated by eight A·A mismatches (Figure 3a).

CD spectra demonstrate that coralyne binds to the **plusA8·minusA8** duplex in a local environment that is the same as, or very similar to, that of the $(dA)_n$ samples (Figure 3b). Job plot analysis based upon coralyne absorption measurements established that coralyne binds to the duplex **plusA8·minusA8** at the level of one coralyne per four adenine bases (Supporting Information), which is suggestive of an intercalative mode of binding within eight A·A base pairs at the level allowed by the nearest-neighbor exclusion principle.[4]

UV melting curves reveal that the T_m of duplex **plusA8·minusA8** increases by 13 °C, from 44 to 57 °C, upon coralyne binding (Figure 3c). These results clearly demonstrate that the homo-adenine−coralyne structure is compatible with the helical structure of a Watson−Crick duplex. As a comparison, the addition of proflavine, a well-characterized intercalator of Watson−Crick duplexes,[5] only stabilizes the **plusA8·minusA8** duplex by 2 °C (T_m = 46 °C) (Figure 3c).

In conclusion, we have presented evidence that an antiparallel duplex can be formed between homo-adenine sequences in the presence of coralyne. We have also demonstrated that this homo-adenine−coralyne structure can be incorporated into duplex structures that include Watson−Crick pairs. Poly(rA) is known to form a duplex with parallel strands and protonated adenine bases under acidic conditions.[6] However, the homo-adenine−coralyne duplex is likely to be significantly different from the low-pH poly(rA) duplex, because the strand orientation can be antiparallel and, as opposed to poly(rA), stability decreases when pH is lowered from neutrality (Supporting Information). Efforts are currently underway in our laboratory to determine the exact structure of the A·A base pairing that exists within the homo-adenine−coralyne duplex. The results presented here are complementary to previous reports that small-molecule binding can be used to alter nucleic acid secondary structure,[7] yet go still further to show that small-molecule binding can even be used to drive the formation of non-Watson−Crick duplexes.

Acknowledgment. We thank L. A. Bottomley for AFM use. This work was supported by grants from the Research Corporation and the National Institutes of Health (GM62873).

Supporting Information Available: Sample preparation details, Job plot analysis, pH and concentration stability studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Mao, C. D.; Sun, W. Q.; Shen, Z. Y.; Seeman, N. C. *Nature* **1999**, *397*, 144−146. (b) Yurke, B.; Turberfield, A. J.; Mills, A. P.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605−608.

(2) Siddiqui-Jain, A.; Grand, C. L.; Bearss, D. J.; Hurley, L. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11593−11598.

(3) (a) Jain, S. S.; Polak, M.; Hud, N. V. *Nucleic Acids Res.* **2003**, *31*, 4608−4615. (b) Polak M.; Hud, N. *Nucleic Acids Res.* **2002**, *30*, 983−992. (c) Ren, J. S.; Chaires, J. B. *Biochemistry* **1999**, *38*, 16067−16075.

(4) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr. *Nucleic Acids. Structures, Properties, and Functions*; University Science Books: Sausalito, 2000.

(5) (a) Shieh, H.-S.; Berman, H. M.; Dabrow, M.; Neidle, S. *Nucleic Acids Res.* **1980**, *8*, 85−97. (b) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450−465.

(6) Rich, A.; Davies, D. R.; Crick, F. H. C.; Watson, J. D. *J. Mol. Biol.* **1961**, *3*, 71−86.

(7) (a) Qu, X.; Trent, J. O.; Fokt, I.; Priebe, W.; Chaires, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12032−12037. (b) Lehrman, E. A.; Crothers, D. M. *Nucleic Acids Res.* **1977**, *4*, 1381−1392. (c) Waring, M. J. *Biochem. J.* **1974**, *143*, 483−486. (d) Hud, N.; Anet, F. A. L. *J. Theor. Biol.* **2000**, *205*, 543−562.

JA0492891