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Coupling across a DNA Helical Turn Yields a Hybrid DNA/Organic Catenane Doubly Tailed with Functional Termini

Yu Liu, Akinori Kuzuya, Ruojie Sha, Johan Guillaume, Risheng Wang, James W. Canary, and Nadrian C. Seeman

Department of Chemistry, New York University, New York, New York 10003

Catenanes constructed from organic building blocks have been used in the demonstration of molecular devices such as positional switches, unidirectional motors, and other nanoscale functional materials.¹ In the nucleic acid world, catenanes have long been known; they are found in nature and are common in structural DNA nanotechnology.² Designed, all-DNA catenanes have been used as topological labels.³ Crosslinks with organic linkages have been utilized to probe the structures and properties of DNA duplexes, hairpins, and higher order structures, such as t-RNA and ribozymes,⁴ as well as to build DNA nanostructures and nanodevices.⁵ In this paper, we describe the synthesis of a macrocycle that is prepared by formation of an amide linkage across one full turn of DNA, forming a tailed DNA/organic catenane containing 5'- and 3'-termini that are available for further functionalization.

In prior work, we showed that 2'-pendent amines and carboxylates could be linked to form nylon along the phosphodiester backbone contour.⁶ Our inquiry here began when we asked whether longer linkers could be used to attach polymeric components parallel to the helix axis. This target requires bridging approximately 35 Å across a nucleic acid turn, which had not been reported previously (Fig. 1A), although there is now a report by Gothelf *et al.*⁷ Tetraethylene glycol was used as a spacer into a carboxylate-functionalized uridine and undecaethylene glycol was incorporated into an aminefunctionalized uridine. The linker distances have not been optimized, although studies with tetraethylene glycol as spacer for both amine and caboxylate were unsuccessful.

Three 16-mer ODNs **1**, **2** and **3** were synthesized, where two modified uridines were separated by 8, 9 and 10 unmodified nucleotides, respectively. Cross-turn coupling using a DNA hairpin template gave > 97% yields of ODN **1** and **2**, as estimated by MALDI-TOF spectra and denaturing gel electrophoresis, whereas the coupling yield of **3** was about 62 % (Table 1, Supporting Information). For further characterization, the coupled product was subjected to complete nuclease digestion followed by analysis to detect linked nucleotides. The detection of a polyethylene glycol (PEG)-amide linked uridine dimer by LCMS corroborated the formation of coupled products (Supporting Information).

These data established the formula of the reaction product but did not distinguish between a newly formed linkage parallel to the helix axis (i.e., across the turn) versus along the phosphodiester backbone contour. However, templation of the reaction by a circular DNA template (Fig. 1A) would yield an interlocked complex only if the coupling reaction occurred across the turn. ODNs **1** and **2** paired with a 78-mer DNA circular template were subjected to

E-mail: E-mail: james.canary@nyu.edu; E-mail: ned.seeman@nyu.edu.

Supporting Information. Full experimental details including: Syntheses of phosphoramidites, MALDI-TOF MS of ODNs, LCMS analysis of complete nuclease digestion, denaturing gel analyses of catenane synthesis, exonuclease digestion and dissociation by restriction enzyme treatment.

the same reaction procedure as the hairpin-templated coupling. Almost-quantitative yields of catenanes were produced from the coupling of both **1** and **2**, which was established by denaturing gel analysis. Thus, the linkage across a full turn of DNA helix generated a doubly tailed catenane with special features discussed below. This demonstrated that the cross-turn coupling strategy produced a linkage parallel to the DNA helix axis.

To test for the catenated structure, the synthesized catenane was first treated with exonuclease; no dissociation was observed, although the tails of the hybrid macrocycle were degraded. To release the DNA hairpin template circle by cleaving the hybrid macrocycle, a catenane was constructed from ODN **4** containing a photocleavable linker (PCL) (Fig. 1B). It was then restricted with BamH I, which cleaved the 78-mer DNA circular template as indicated in Fig. 1B to produce an 18-mer and a 60-mer and released the PEG-amide-oligonucleotide hybrid circle as shown by its electrophoretic mobility (Fig. 2, lane 6). Identical products were obtained from cleavage of the DNA template alone (Fig. 2, lane 7) and coupled ODN **4** (lane 8). Upon irradiating the catenane with near-UV light (350 nm), the PCL was cleaved, resulting in linearization of the hybrid macrocycle and release of template ring. The reaction products appear in Fig. 2 (lane 4), and can be compared with intact catenane and DNA hairpin template (lanes 5 and 3), and photocleavage products of coupled strand **4** (lane 2).

The catenane formation reaction may be viewed as a “padlock” function as the oligonucleotide acted as a probe to recognize the target circular DNA and interlocking was accomplished by the pendent linkers. The 2'-localization of the linkers creates a bibrachial lariat structure,⁸ leaving the free 5'- and 3'-ends of the oligonucleotides available to serve as accessible sites for further modification or labeling, which is an advantage over prior approaches that employ 5'- and 3'-terminal linkers.^{5a,9} To test the feasibility of postsynthetic modification of these tails, the catenane containing ODN **4** was subjected to 5'- and 3'-³²P labeling in two experiments. The generation of 3'-labeled product (Fig. 3, lane 1) and of 5'-labeled product (lane 3) are readily visible in the autoradiogram of the denaturing gel. Thus, this cross-turn coupling strategy may be advantageous for nucleic acid labeling. In principle, nucleic acids of any sequence could be targeted and labeled by this approach.

In conclusion, coupling across a DNA helical turn was achieved utilizing ODNs decorated with 2'-functionalized linkers. The formation of a doubly tailed catenane based on this strategy demonstrates intra-strand crosslinking. The free ends of the catenated PEG-amide-oligonucleotides provide useful handles for post-synthetic modification or labeling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Arico F, Badjic JD, Cantrill SJ, Flood AH, Leung KCF, Liu Y, Stoddart JF. Topics in Current Chemistry 2005;249(Templates in Chemistry II) b Champin B, Mobian P, Sauvage JP. Chem. Soc. Rev 2007;36:358–366. [PubMed: 17264936] c Kay ER, Leigh DA, Zerbetto F. Angew. Chem., Int. Ed 2007;46:72–191. d Lankshear MD, Beer PD. Acc. Chem. Res 2007;40:657–668. [PubMed: 17523590]
2. Seeman, NC. Synthetic DNA Topology, Molecular Catenanes, Rotaxanes and Knots. Sauvage, J-P.; Dietrich-Buchecker, D., editors. Wiley-VCH; Weinheim: 1999. p. 323-356.

3. Liang XG, Kuhn H, Frank-Kamenetskii MD. *Biophys. J* 2006;90:2877–2889. [PubMed: 16461397]
4. a El-Sagheer AH, Kumar R, Findlow S, Werner JM, Lane AN, Brown T. *Chembiochem* 2008;9:50–52. [PubMed: 18058775] b Walter NG, Burke JM. *Curr. Opin. Chem. Biol* 1998;2:24–30. [PubMed: 9667918] c Glick GD. *Biopolymers* 1998;48:83–96. [PubMed: 9846126]
5. a Kumar R, El-Sagheer A, Tumpene J, Lincoln P, Wilhelmsson LM, Brown T. *J. Am. Chem. Soc* 2007;129:6859–6864. [PubMed: 17488075] b Endo M, Uegaki S, Majima T. *Chem. Commun* 2005:3153–3155. c Endo M, Seeman NC, Majima T. *Angew. Chem. Int. Ed* 2005;44:6074–6077.
6. a Zhu L, Lukeman PS, Canary JW, Seeman NC. *J. Am. Chem. Soc* 2003;125:10178–10179. [PubMed: 12926933] b Liu Y, Wang R, Ding L, Sha R, Lukeman PS, Canary JW, Seeman NC. *Chembiochem* 2008;9:1641–1648. [PubMed: 18543259] c Liu Y, Sha R, Wang R, Ding L, Canary JW, Seeman NC. *Tetrahedron*. in press
7. Andersen CS, Yan H, Gothelf K. *Angew. Chemie*. in press
8. Gokel GW, Barbour LJ, Ferdani R, Hu J. *Acc Chem Res* 2002;35:878–886. [PubMed: 12379140]
9. a Ryan K, Kool ET. *Chem. Biol* 1998;5:59–67. [PubMed: 9495829] b Escude C, Garestier T, Helene C. *Proc. Natl. Acad. Sci. USA* 1999;96:10603–10607. [PubMed: 10485872] c Demidov VV, Kuhn H, Lavrentieva-Smolina IV, Frank-Kamenetskii MD. *Methods* 2001;23:123–131. [PubMed: 11181031]

Figure 1. Schematic depiction of (A) templated synthesis of the tailed catenane, (B) cleavage, and labeling of the tailed catenane constructed from strand **4**. The catenane stereoisomer is established by the antiparallel pairing of DNA before closure.

Figure 2.

Denaturing gel analysis of the dissociation of the tailed catenane by BamH I digestion and UV cleavage. Lane 1: 10bp DNA ladder marker, Lane 2: UV-cleaved products of coupled strand 4, Lane 3: 78-mer DNA circle, Lane 4: UV-cleaved products of the tailed catenane, Lane 5: The tailed catenane, Lane 6: BamH I digested products of the tailed catenane, Lane 7: BamH I digested products of 78-mer DNA circle, Lane 8: Coupled strand 4, Lane 9: Uncoupled strand 4. L: Linear DNA, C: Circular DNA.

Figure 3.

Autoradiogram of a denaturing gel showing both 5' and 3' labeling of the tailed catenane. Lane 1: 3'-³²P labeling of the tailed catenane. Lane 2: 3'-³²P labeling of 78-mer DNA circle. Lane 3: 5'-³²P labeling of the tailed catenane. Lane 4: 5'-³²P labeling of 78-mer DNA circle. Lane 5: 10bp DNA ladder marker.

Table 1
Oligonucleotides used in this study and MALDI-TOF MS analysis. ^a

ODNs	Sequence	Uncoupled		Coupled	
		Calc.	Found	Calc.	Found
1	5'-TG U ³⁶ _N ACGTGC GA U ¹⁶ _C TTTCG	5729.2	5729.4	5711.2	5711.3
2	5'-TG U ³⁶ _N ACGTGC GA U ¹⁶ _C TCG	5729.2	5728.4	5711.2	5710.5
3	5'-TG U ³⁶ _N ACGTGC GA U ¹⁶ _C CG	5729.2	5728.9	5711.2	5711.0, 5728.1
4	5'-TG U ³⁶ _N ACGTG(PCL)CGAT U ¹⁶ _C TCG ^b	5988.4	5988.4	5970.4	5969.1

^a Cross-turn coupling trials were conducted with a DNA hairpin as template.

^b PCL: Photocleavable linker.