Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2233–2237, March 1998
Biochemistry

Cleaving DNA with DNA

NIR CARMI, SHAMEELAH R. BALKHI, AND RONALD R. BREAKER†

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103

Edited by Charles R. Cantor, Boston University, Boston, MA, and approved December 23, 1997 (received for review November 3, 1997)

ABSTRACT **A DNA structure is described that can cleave single-stranded DNA oligonucleotides in the presence of ionic copper. This "deoxyribozyme" can self-cleave or can operate as a bimolecular complex that simultaneously makes use of duplex and triplex interactions to bind and cleave separate DNA substrates. Bimolecular deoxyribozyme-mediated strand scission proceeds with a k_{obs} of 0.2 min^{-1}, whereas the corresponding uncatalyzed reaction could not be detected. The duplex and triplex recognition domains can be altered, making possible the targeted cleavage of single-stranded DNAs with different nucleotide sequences. Several small synthetic DNAs were made to function as simple "restriction enzymes" for the site-specific cleavage of single-stranded DNA.**

DNA in biological systems exists primarily in duplex form where it serves almost exclusively as a storage system for genetic information. Outside the confines of cells, DNA in its single-stranded form can be made to perform both molecular recognition and catalysis—biochemical operations that were until recently thought to be possible only with macromolecules made of protein or RNA. For example, a number of DNA "aptamers" (1) can be made that function as ligands for proteins or as highly specific receptors for small organic molecules (2–4). In addition, certain single-stranded DNAs act as artificial enzymes (4–6), catalyzing such chemical reactions as phosphoester transfer (7–12), phosphoester formation (13), porphyrin metalation (14), phosphoramidate cleavage (15), and DNA cleavage (16). Most likely, DNA can be made to perform a much broader repertoire of catalytic activities (6).

These capabilities of DNA conceivably can be exploited to create a variety of structured DNAs that perform useful tasks, either *in vitro* or *in vivo*, involving molecular recognition and catalysis. Such functional DNAs offer several advantages, including ease of synthesis and chemical stability, that might make attractive properties for polymers that serve as artificial receptors or as biocatalysts for various applications. Characteristics such as thermostability and solvent/solute preferences could be conferred upon deoxyribozymes by using both rational and combinatorial methods of molecular design. Catalytic DNAs may offer distinct advantages over natural protein enzymes for operation under nonbiological conditions.

In a previous study (16), we reported the isolation of two distinct types of deoxyribozymes (classes I and II) that undergo oxidative self-cleavage in the presence of copper ions. By using *in vitro* selection (17), class II self-cleaving DNAs have been further optimized for catalytic function, and the most active structure obtained from this process has been engineered to act as a "restriction endonuclease" for single-stranded DNA substrates.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Oligonucleotides. Synthetic DNAs were prepared by automated chemical synthesis (Keck Biotechnology Resource Laboratory, Yale University) and were purified by denaturing (8 M urea) PAGE prior to use. Double-stranded 101-mer DNA was prepared by PCR as described (16) with the primer DNAs 5′-[^{32}P]GTCGACCTGCGAGCTCGA, 5′-GTAGATCGTAAAGCTTCG, and the 101-mer DNA oligomer (see Fig. 4*A*) as template.

***In Vitro* Selection.** Optimization of class II self-cleaving DNAs was achieved by *in vitro* selection essentially as described (16) by using a reaction mixture for DNA cleavage composed of 50 mM Hepes (pH 7.0 at 23°C), 0.5 M NaCl, and 0.5 M KCl (buffer A) containing 10 μM $CuCl_2$. The selection process was initiated with 20 pmol of G7 PCR DNA (16) in which the 5′ terminus of each catalyst strand carried a biotin moiety, thereby allowing DNA from this and subsequent generations to be immobilized on a streptavidin-derivatized chromatographic matrix. Reaction time was 15 min for immobilized DNA from G8–G10 and 12, 7, and 5 min for the G11–G13 DNA populations, respectively. Individual self-cleaving DNAs from G13 were analyzed by cloning (Original TA cloning kit, Invitrogen) and sequencing (Sequenase 2.0 DNA sequencing kit, U.S. Biochemicals).

DNA Cleavage Assays. To assess the DNA cleavage activity of self-cleaving molecules, radiolabeled precursor DNA was prepared by enzymatically tagging the 5′ terminus of synthetic DNAs in a reaction containing 25 mM 2-(*N*-cyclohexylamino)-ethanesulfonic acid (CHES; pH 9.0 at 23°C), 5 mM $MgCl_2$, 3 mM DTT, 1 μM DNA, 1.2 μM [γ-^{32}P]ATP (~130 μCi; 1 Ci = 37 GBq), and T4 polynucleotide kinase (1 unit/μl), which was incubated at 37°C for 1 hr. The resulting 5′-^{32}P-labeled DNA was isolated by denaturing PAGE and recovered from the gel matrix by crush-soaking in 10 mM Tris·HCl (pH 7.5 at 23°C), 0.2 M NaCl, and 1 mM EDTA. The recovered DNA was concentrated by precipitation with ethanol and resuspended in deionized water (Milli-Q, Millipore). Self-cleavage assays using trace amounts of radiolabeled precursor DNA (~100 pM) were conducted at 23°C in buffer A containing $CuCl_2$ as indicated for each experiment. Examinations of the DNA cleavage activity of bimolecular complexes were conducted under similar conditions with trace amounts of 5′-^{32}P-labeled "substrate" DNA. Cleavage products were separated by denaturing PAGE and imaged by autoradiography or by PhosphorImager (Molecular Dynamics), and product yields were determined by quantitation (IMAGEQUANT) of the corresponding precursor and product bands.

Kinetic Analyses. Catalytic rates were estimated by plotting the fraction of precursor or substrate DNA cleaved versus time and establishing the slope of the curve that represents the initial velocity of the reaction as determined by a least-squares fit to the data. Upon close examination, DNA cleavage in both the substrate and enzyme domains displayed a brief lag phase that complicates the determination of the initial cleavage rate (unpublished observations). To avoid the lag phase, the initial

This paper was submitted directly (Track II) to the *Proceedings* office.
†To whom reprint requests should be addressed.

slope was calculated by using only data collected after the reaction had proceeded for 1 min. Rates obtained from replicate experiments differed by less than 50% and the values reported are averages of at least three analyses.

RESULTS AND DISCUSSION

A Minimal Cu^{2+}-Dependent Self-Cleaving DNA. In a previous study, a variety of self-cleaving DNAs were isolated by *in vitro* selection from a pool of random-sequence molecules (16). Most individual DNAs that were isolated after eight rounds (G8) of selection conformed to two distinct classes, based on similarities of nucleotide sequence and DNA cleavage patterns. Although individual DNAs from both class I and class II require Cu^{2+} and ascorbate for full activity, the G8 DNA population displays weak self-cleavage activity in the presence of Cu^{2+} alone. A representative class II DNA termed CA3 was further optimized for ascorbate-independent activity by applying *in vitro* selection to a DNA pool that was composed of mutagenized CA3 molecules. The sequence data from this artificial phylogeny of DNAs indicates that as many as 27 nucleotides, most of them located near the 3′ terminus of the molecule, are important for self-cleavage activity.

Beginning with the original G7 DNA population, we carried out an additional six rounds of *in vitro* selection for DNAs that self-cleave in the presence of 10 μM Cu^{2+}, without added reducing agent. Analysis of the G13 population of DNAs revealed robust self-cleavage activity (data now shown), demonstrating that catalytic DNAs can promote efficient cleavage of DNA by using only a divalent metal cofactor. The G13 population displays the same cleavage pattern that was observed with individual class II DNAs, indicating that class II-like deoxyribozymes dominate the final DNA pool.

A total of 27 individual DNAs from G13 were sequenced (data not shown), and without exception, each carried a 21-nucleotide sequence domain that largely conformed to the consensus sequence that was used previously to define class II self-cleaving DNAs. Although individuals that have a strictly conserved core (spanning nucleotides 11–31, Fig. 1*A*) dominate the G13 pool, two common variations from this consensus sequence include a C → T mutation at position 17 (6 of 28 individuals) or the presence of six successive thymidines instead of five in the region spanning nucleotides 21–25 (4 of 27 individuals). However, significant differences in nucleotide sequence were found to occur outside this conserved domain, indicating that large portions of the class II deoxyribozymes isolated may not be necessary for catalytic activity. Indeed, three individual DNAs were found to have undergone deletions of 16, 19, and 20 nucleotides within the 50-nucleotide domain that was randomized in the original starting pool. The predicted secondary structure for the 19-nucleotide deletion mutant (69-mer DNA, Fig. 1*A*), obtained by the Zuker DNA MFOLD program‡ (18, 19), indicates the presence of three base-paired regions; two involve pairing between the original random-sequence domain and the "substrate" domain, and one involves putative base pairing of nucleotides that lie within the conserved-sequence region. A synthetic DNA corresponding to the 69-mer depicted in Fig. 1*A* undergoes Cu^{2+}-dependent self-cleavage at two locations with an overall rate constant of 0.3 min^{-1} under the conditions used for *in vitro* selection (see *Materials and Methods* for additional discussion on catalytic rates).

We tested whether the two pairing regions of the 69-mer that lie within the variable-sequence region could be replaced by a smaller stem–loop structure by synthesizing a 46-mer DNA, in which 26 nucleotides of this imperfect hairpin were replaced by

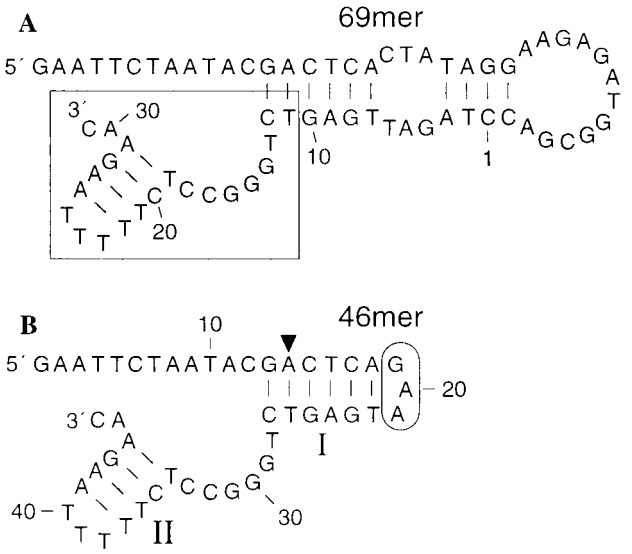

FIG. 1. Sequence and predicted secondary structures of minimized self-cleaving DNAs. (*A*) Sequence and secondary structure of a synthetic 69-nucleotide self-cleaving DNA that was isolated by *in vitro* selection. Numbers identify nucleotides that correspond to the 50-nucleotide random-sequence domain that was included in the original DNA pool (note that 19 bases of this domain have been deleted). The conserved nucleotides (nucleotides 11–31, boxed) are similar to those previously used to define this class of deoxyribozymes (16). (*B*) A 46-nucleotide truncated version of class II DNAs that retains full activity. **I** and **II** designate stem–loop structures of the 46-mer that are predicted by the structural folding program DNA MFOLD (18, 19) and that were confirmed by subsequent mutational analysis (Fig. 2). The conserved core of the deoxyribozyme spans nucleotides 27–46 and the major site of DNA cleavage is designated by the arrowhead. Encircled nucleotides can be removed to create a bimolecular complex where nucleotides 1–18 constitute the substrate subdomain, and nucleotides 22–46 constitute the catalyst subdomain.

the trinucleotide loop GAA (Fig. 1*B*). As expected, the truncated 46-mer DNA retains full catalytic activity, thereby confirming that the deleted nucleotides are not essential for deoxyribozyme function. This 46-nucleotide deoxyribozyme is predicted to adopt a pistol-like secondary structure (Fig. 1*B*) composed of two base-paired structural elements (stems I and II) flanked by regions of single-stranded DNA. The primary site of DNA cleavage is located at position 14 that resides within one of the putative stem structures of the 46-mer. The catalyst also promotes DNA cleavage within a region located apart from the main cleavage site (16), as might be expected for a deoxyribozyme that makes use of an oxidative cleavage mechanism (20). Details from the further characterization of class II deoxyribozyme function will be reported elsewhere (N.C., R. Baliga, D. M. Crothers, and R.R.B., unpublished data).

Bimolecular Deoxyribozyme Complexes: Substrate Recognition by Duplex and Triplex Formation. Separate "substrate" and "catalyst" DNAs can be created from the 46-mer by eliminating the connecting loop of stem I (Fig. 1*B*). Active bimolecular complexes then can be reconstituted by combining independently prepared substrate and catalyst DNAs. Both the unimolecular 46-mer and the bimolecular complexes examined cleave with identical rates, promoting primary-site cleavage with a k_{obs} of approximately 0.2 min^{-1}. The importance of stem I was confirmed (Fig. 2*A*) by synthesizing different catalyst DNAs (c1, c2, and c3) and assessing their ability to cleave different substrate molecules (s1, s2, and s3). For example, c1 displays activity with its corresponding substrate (s1) but not when the noncomplementary substrate DNAs s2 or s3 are substituted. Likewise, c2 and c3 only cleave their corresponding substrate DNAs s2 and s3, respectively. Extending stem I

‡The DNA mfold server can be accessed on the internet (www.ibc.wustl.edu/~zuker/dna/form1.cgi).

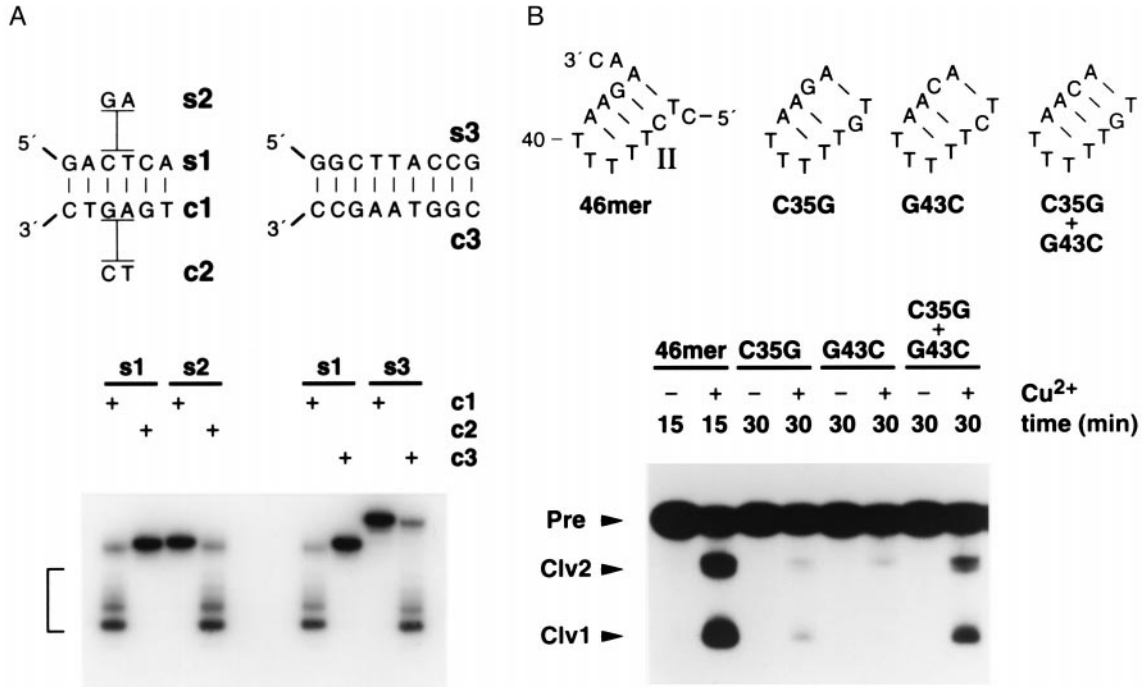

FIG. 2. Confirmation of stems I and II by mutational analysis. (*A*) Trace amounts of 5′-^{32}P-labeled substrate DNAs (s1–s3) were incubated with 5 μM complementary or noncomplementary catalyst DNAs (c1–c3) in reaction buffer A containing 10 μM $CuCl_2$ at 23°C for 15 min. Reaction products were separated by denaturing PAGE on 20% gels and imaged by autoradiography. Bracket identifies the position of the substrate cleavage products. (*B*) Self-cleavage activity of the original 46-mer sequence compared with the activity of variant DNAs with base substitutions in stem II. Individual 46-mer variants (100 pM 5′-^{32}P-labeled precursor DNA) were incubated for the times indicated under reaction conditions as described above. Clv1 and Clv2 identify 5′ cleavage fragments produced upon precursor DNA (Pre) scission at the primary and secondary sites, respectively. Mutated positions use the numbering system given in Fig. 1*B*.

to create a more stable interaction was also found to confer greater binding affinity between substrate and catalyst oligonucleotides (N.C., R. Baliga, D. M. Crothers, and R.R.B., unpublished data). These data indicate that base-pairing interactions that constitute stem I are an essential determinant for catalyst–substrate recognition.

Stem II was examined by a similar approach using mutant versions of the 46-mer self-cleaving DNA. A series of variant deoxyribozymes with one or two mutations included in the putative stem II structure were synthesized and assayed for catalytic activity (Fig. 2*B*). Disruption of the original C35·G43 base pair in stem II, either by mutation of C → G at position 35 or mutation of G → C at position 43, results in a substantial loss of activity. Cleavage activity is partially restored when these mutations are combined in the same molecule to produce a G35·C43 base pair. These results are consistent with the stem–loop structure modeled in Fig. 1. Additional support for the presence of stem II was found upon sequence analysis of the deoxyribozymes that are present in the original *in vitro*-selected pool of DNAs. A single self-cleaving DNA was found with a core sequence that differs significantly from that of the most frequently represented deoxyribozyme (N.C., R. Baliga, D. M. Crothers, and R.R.B., unpublished data). Nucleotides 38–40 of the more common 46-mer sequence are replaced in the variant deoxyribozyme with the nucleotides 5′-CTGGGG. This alternative sequence extends stem II by a single C·G base pair, consistent with the formation of the predicted stem–loop structure.

Although the existence of stem II is supported by the data derived from mutational analysis, the fact that total restoration of deoxyribozyme activity was not achieved with restoration of base complementation indicates that the identities of the base pairs in this structural element are important for maximal catalytic function. Moreover, we find that mutation or deletion of nucleotides 1–7 of the 46-mer result in a dramatic loss of DNA cleavage activity. We recognized that nucleotides 4–7 within this essential region of the substrate form a polypyrimidine tract that is complementary to the paired sequence of stem II for the formation of a YR*Y DNA triple helix (21).

To examine the possibility of triplex formation in the active structure of the deoxyribozyme, we modified both the base-pairing sequence of stem II (c4) and the sequence of the polypyrimidine tract of the substrate (s4) to alter the specificity yet retain the potential for forming four contiguous base triples (Fig. 3). The c4 variant DNA cleaves its corresponding s4 DNA substrate but shows no activity with a substrate that carries the original polypyrimidine sequence. We find that even single mutations within stem II (e.g., Fig. 2*B*) or single mutations within the polypyrimidine tract cause significant reductions in catalytic activity. However, the introduction of six mutations in a manner that is consistent with triplex formation results in a variant (c4–s4) complex that displays full DNA cleavage activity. This is the only example of a catalytic polynucleotide,

FIG. 3. Identification of a triplex interaction between substrate and catalyst DNAs. A revised structural representation portrays a triple-helix interaction (dots) between the four base pairs of stem II and four consecutive pyrimidine residues near the 5′ end of the substrate DNA. c4 and s4 represent sequence variants of c3 and s3 that retain base pairing within stem II and that use an alternate sequence of base triples. DNA cleavage assays were conducted as described in Fig. 2*A*. Bracket identifies the position of the substrate cleavage products.

natural or artificial, that makes use of an extended triple helix for the formation of its active structure (22).

Targeted Cleavage of DNA Restriction Sites with Deoxyribozymes. The results described above demonstrate that class II deoxyribozymes identify substrate DNAs by simultaneously using two distinct recognition domains that are formed separately by stems I and II. We reasoned that these structures might be further exploited as recognition elements to engineer deoxyribozymes that selectively cleave DNAs at different target sites. To demonstrate this capability, we synthesized a 101-nucleotide DNA that carries three identical leader sequences, each followed by a different stem I recognition sequence (Fig. 4*A*). Three catalyst DNAs (c1, c3, and c7) were designed to be uniquely complementary to one of the three target sites. When incubated separately with 101-mer substrate, DNAs c3 and c7 cleave exclusively at their corresponding target sites, whereas c1 cleaves at its intended site and also to a lesser extent at the c3 cleavage site (Fig. 4*B*). The cross-reactivity observed with c1 can be explained by examining the base-pairing potential of stem I. Of the six nucleotides in the c1 recognition sequence, four can form standard base pairs, whereas the remaining two form G·T wobble pairs. The contribution of both duplex and triplex recognition elements presumably allows for detectable cleavage activity at this secondary location.

The triplex interaction that is defined by the base-pairing sequence of stem II can also be exploited to target specific DNA substrates. We designed three new catalyst DNAs (c9, c10, and c11) that carry identical stem I pairing subdomains but that have expanded and unique stem II subdomains (Fig. 4*C*). When incubated separately with a 100-nucleotide DNA that carries three uniquely complementary polypyrimidine tracts, each catalyst DNA cleaves its corresponding target site with a rate that corresponds well with that found for the original self-cleaving DNA. In this example, substrate selectivity is determined almost entirely by triplex formation, despite the presence of identical and extensive base complementation (stem I) between catalyst and substrate molecules.

Although DNA cleavage catalyzed by the deoxyribozyme is focused within the substrate domain, substantial (~30%) cleavage occurs within the conserved core of the catalyst strand. This collateral damage causes inactivation of the deoxyribozyme and, as a result, superstoichiometric amounts of catalyst DNA are needed to assure quantitative cleavage of DNA substrate. Cleavage of the substrate subdomain proceeds more rapidly than does cleavage within the catalytic core. In the presence of excess c1, s1 is cleaved at a rate of approximately 0.2 min^{-1} (reaction buffer containing 30 μM $CuCl_2$), reaching a plateau of ~80% cleaved after 20 min. In contrast, cleavage of c1 in the presence of excess s1 proceeds ~2-fold slower, consistent with our earlier report that the ratio of self-cleavage localized in the substrate domain to self-cleavage in the catalytic core gives a ratio of ~2:1 (16). We have established that, barring inactivation by miscleavage, the catalyst strand can undergo multiple turnovers (N.C., R. Baliga, D. M. Crothers, and R.R.B., unpublished data).

Thermal Denaturation of Double-Stranded DNA Targets. Class II catalyst DNAs are not able to cleave target DNAs that

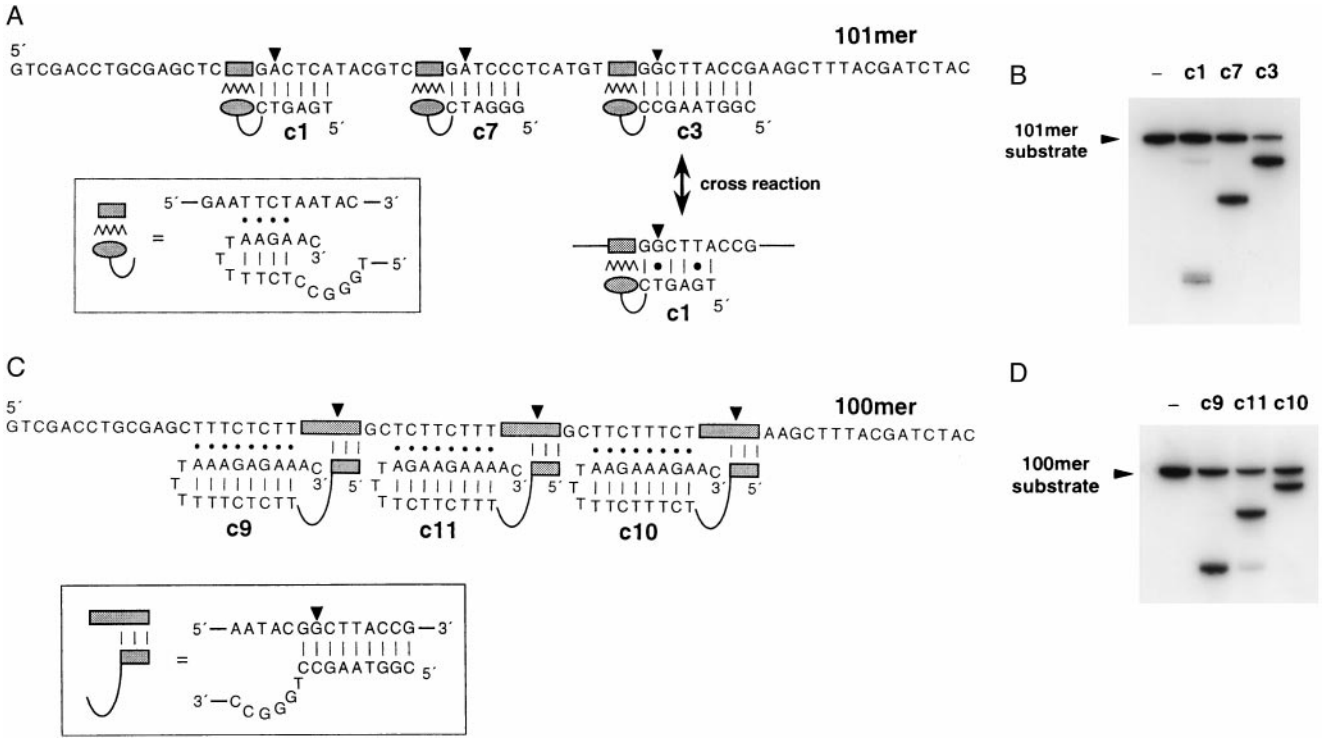

FIG. 4. Targeted cleavage of DNA substrates with deoxyribozymes containing engineered duplex and triplex recognition elements. (*A*) A 101-nucleotide DNA incorporating three different deoxyribozyme cleavage sites was prepared by automated chemical synthesis. Each cleavage site consists of an identical leader sequence (shaded boxes) followed by a stem I recognition element of unique sequence. The specific base complementation between the synthetic catalyst DNAs c1, c3, and c7 is also depicted. The catalytic core sequences and the leader sequence–stem II interactions for each site are identical (*Inset*). Large dots indicate G·T wobble pairs that allow cross-reaction between c1 and the target for c3. Small dots indicate base triple interactions. (*B*) Cleavage of the 101-mer DNA by c1, c3, and c7 was examined by incubating trace amounts of 5′-^{32}P-labeled substrate in reaction buffer containing 30 μM $CuCl_2$ at 23°C for 20 min in the absence (−) or presence of 5 μM catalyst DNA, as indicated. Reaction products were separated by denaturing PAGE on 10% gels and visualized by autoradiography. (*C*) Similarly, a 100-nucleotide DNA was prepared that contained three identical stem I pairing regions (shaded boxes) preceded by eight successive pyrimidine nucleotides of unique sequence. Three synthetic deoxyribozymes (c9, c10, and c11) that carry identical stem I pairing elements (*Inset*) and extended stem II subdomains of unique sequence were designed to target the three cleavage sites exclusively through DNA triplex interactions. (*D*) Cleavage of 100-mer DNA by c9, c10, and c11 was established as described in *B* above. Miscleavage is detected for each triplex-guided deoxyribozyme upon extended exposure during autoradiography (e.g., c11), indicating that weak-forming triplex interactions allow some DNA cleavage activity to occur.

reside within a duplex. The catalyst DNA, with its short recognition sequence, presumably cannot displace the longer and more tightly bound complementary strand of the target to gain access to the cleavage site. We have found that an effective means for specific cleavage of one strand of an extended DNA duplex makes use of repetitive cycles of thermal denaturation and reannealing. For example, c3 remains inactive against a double-stranded DNA target in the absence of thermal cycling but efficiently cleaves the same DNA substrate upon repeated heating and cooling cycles (Fig. 5). Cleavage of the radiolabeled target is quantitative after six thermal cycles. DNA cleavage by class II DNAs occurs within the base-pairing region corresponding to stem I, presumably when this region is in double-helical form. This, coupled with the observation of substrate recognition by triplex formation, suggests that different DNA enzymes might be engineered to cleave duplex DNA substrates without the need for thermal denaturation. Such deoxyribozyme activity would be similar to that performed by a number of triplex-forming oligonucleotides that have been engineered to bind and cleave duplex DNA by using a chemically tethered metal complex such as Fe-EDTA (23–26) or Cu-phenanthroline (27).

Conclusions. In its unimolecular arrangement, the class II deoxyribozyme could be used to confer the capacity for self-destruction to an otherwise stable DNA construct. In its bimolecular form, the deoxyribozyme can act as an artificial restriction enzyme for single-stranded DNA, whereas protein-based nucleases that cleave nonduplex DNA do not demonstrate significant sequence specificity. It is likely that maximal discrimination by class II catalysts between closely related target sequences can be achieved through careful design of the duplex and triplex recognition domains. This is expected to eliminate the cross reactivity that was observed in our studies. Although the role of most nucleotides within the substrate domain are involved in substrate recognition, the importance of each nucleotide within the leader sequence has yet to be fully delineated. However, guided by the basic rules of duplex and triplex formation, we can now engineer highly specific deoxyribozymes that can catalyze the cleavage of single-stranded DNA at defined locations along a polynucleotide chain.

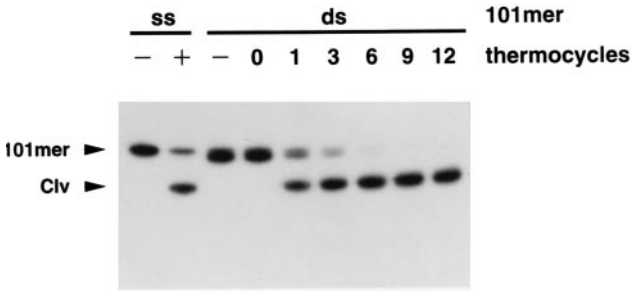

FIG. 5. Cleaving double-stranded DNA. Trace amounts of single-stranded (ss) or double-stranded (ds) 101-mer DNA (target strand is 5′-^{32}P-labeled) was incubated in buffer A containing 10 μM $CuCl_2$ either without (−) or with (+) 5 μM c3. Single-stranded DNA and the first lane of double-stranded DNA was incubated at room temperature without thermocycling for 15 min. The remainder of the double-stranded DNA samples were incubated in the presence of 5 μM c3 either without (0) or with thermal denaturation (1–12 cycles) as indicated. Thermocycling consisted of repetitive heating and cooling between 94°C (1 min) and 25°C (15 min). Numbers indicate the iterations of thermal cycling and Clv identifies the DNA cleavage product. Reaction products were separated by denaturing PAGE in 10% gels and visualized by autoradiography.

We thank G. F. Joyce for comments on the manuscript and members of the Breaker laboratory for helpful discussions. This work was supported by a Young Investigator Award to R.R.B. from the Arnold and Mabel Beckman Foundation.

1. Ellington, A. D. & Szostak, J. W. (1990) *Nature (London)* **346,** 818–822.
2. Gold, L., Poliski, B., Uhlenbeck, O. & Yarus, M. (1995) *Annu. Rev. Biochem.* **64,** 763–797.
3. Osborne, S. E. & Ellington, A. D. (1997) *Chem. Rev.* **97,** 349–370.
4. Breaker, R. R. (1997) *Curr. Opin. Chem. Biol.* **1,** 26–31.
5. Burgstaller, P. & Famulok, M. (1995) *Angew. Chem. Int. Ed. Engl.* **34,** 1189–1192.
6. Breaker, R. R. (1997) *Nat. Biotechnol.* **15,** 427–431.
7. Breaker, R. R. & Joyce, G. F. (1994) *Chem. Biol.* **1,** 223–229.
8. Breaker, R. R. & Joyce, G. F. (1995) *Chem. Biol.* **2,** 655–660.
9. Santoro, S. W. & Joyce, G. F. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4262–4266.
10. Faulhammer, D. & Famulok, M. (1996) *Angew. Chem. Int. Ed. Engl.* **35,** 2837–2841.
11. Faulhammer, D. & Famulok, M. (1997) *J. Mol. Biol.* **269,** 188–203.
12. Geyer, C. R. & Sen, D. (1997) *Chem. Biol.* **4,** 579–593.
13. Cuenoud, B. & Szostak, J. W. (1995) *Nature (London)* **375,** 611–614.
14. Li, Y. & Sen, D. (1996) *Nat. Struct. Biol.* **3,** 743–747.
15. Burmeister, J., von Kiedrowski, G. & Ellington, A. D. (1997) *Angew. Chem. Int. Ed. Engl.* **36,** 1321–1324.
16. Carmi, N., Shultz, L. A. & Breaker, R. R. (1996) *Chem. Biol.* **3,** 1039–1046.
17. Breaker, R. R. (1997) *Chem. Rev.* **97,** 371–390.
18. Zuker, M. (1989) *Science* **244,** 48–52.
19. SantaLucia, J., Jr., Allawi, H. T. & Seneviratne, P. A. (1996) *Biochemistry* **35,** 3555–3562.
20. Joshi, R. R., Likhite, S. M., Kumar, R. K. & Ganesh, K. N. (1994) *Biochim. Biophys. Acta* **1199,** 285–292.
21. Frank-Kamenetskii, M. D. & Mirkin, S. M. (1995) *Annu. Rev. Biochem.* **64,** 65–95.
22. Strobel, S. A. & Doudna, J. A. (1997) *Trends Biochem. Sci.* **22,** 262–266.
23. Moser, H. E. & Dervan, P. B. (1987) *Science* **238,** 645–650.
24. Beal, P. A. & Dervan, P. B. (1991) *Science* **251,** 1360–1363.
25. Strobel, S. A. & Dervan, P. B. (1990) *Science* **249,** 73–75.
26. Strobel, S. A. & Dervan, P. B. (1992) *Methods Enzymol.* **216,** 309–321.
27. Francois, J.-C., Saison-Behmoaras, T., Barbier, C., Chassignol, M., Thuong, N. T. & Helene, C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9702–9706.