J|A|C|S
COMMUNICATIONS

Published on Web 02/14/2008

DNA and RNA Can Be Equally Efficient Catalysts for Carbon−Carbon Bond Formation

Madhavaiah Chandra and Scott K. Silverman*

Department of Chemistry, University of Illinois at Urbana−Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801

Received December 17, 2007; E-mail: scott@scs.uiuc.edu

Catalysis by nucleic acids was merely a theoretical possibility until the discovery of catalytic RNAs (ribozymes) in the early 1980s.[1] Although a variety of natural ribozymes have since been identified,[2] analogous catalytic DNAs (deoxyribozymes) have not been found in nature. In the laboratory, many artificial ribozymes and deoxyribozymes have been identified through in vitro selection by starting with pools of random sequences.[3] The repertoire of artificial ribozymes discovered in this fashion encompasses many chemical reactions including phosphodiester cleavage and ligation,[4] RNA polymerization,[5] redox reactions,[6] carbon−carbon bond formation (Diels−Alder reaction),[7,8] and many others.[9] Because DNA catalysts were identified later than RNA[10] and because natural ribozymes provide a strong motivation to study artificial RNA catalysts, the catalytic abilities of DNA have not been examined as thoroughly as those for RNA.[11] An early speculation was that the lack of 2′-hydroxyl groups in DNA would likely impair its catalytic efficiency relative to RNA,[12] providing a specific concern about the functional range of DNA as a catalyst. The available data for the most commonly studied DNA-catalyzed reaction, RNA cleavage,[13] suggest that RNA and DNA should be equally competent, although in both cases the highest theoretically possible rate enhancements have likely not been achieved.[14] In this report we investigated DNA catalysis of the Diels−Alder reaction, anticipating that the results would allow a clear comparison between the catalytic efficiencies of artificial ribozymes and deoxyribozymes for this important carbon−carbon bond-forming reaction. We identified deoxyribozymes that can catalyze the Diels−Alder reaction as efficiently as the reported ribozymes, providing evidence that DNA can be as catalytically efficient as RNA for C−C bond formation.[15]

We began by considering the Diels−Alder ribozyme that was identified by Jäschke and co-workers using in vitro selection.[8] This ribozyme catalyzes the bimolecular Diels−Alder reaction between suitably functionalized anthracene and maleimide substrates with multiple turnover. The structural basis for catalysis has been elucidated through X-ray crystallography,[16] and the scope of substrate tolerance has been explored.[17] One minimal form of this Diels−Alder ribozyme, 39M49, has 49 nucleotides.[8] With 39M49 in mind, we arranged two parallel deoxyribozyme selection experiments. In the first selection experiment, designated "DAR" for "Diels−Alder Random", we used an entirely random 40-nucleotide (N_{40}) sequence pool. In the second selection experiment, designated "DAB" for "Diels−Alder Biased", we used a biased (i.e., partially randomized) pool that was derived from the 39M49 ribozyme sequence but synthesized as DNA, with 36 nucleotides of the sequence partially randomized. Each of these 36 nucleotides had 70% probability of having the original nucleotide identity and 10% probability each of having the other three possible identities. On this basis, the mean number of nucleotide differences between the 39M49 ribozyme and an arbitrary DNA sequence from the biased pool is ca. 11 nucleotides (30% of 36 = 10.8), although a wide range of mutations per sequence is statistically represented.

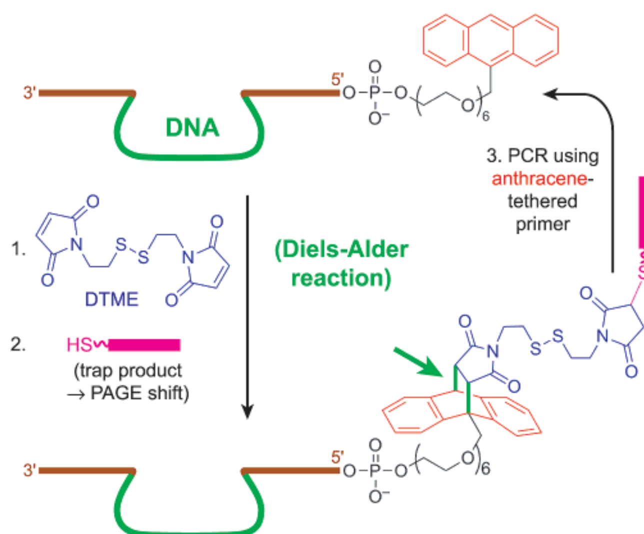

Figure 1. Strategy for in vitro selection of deoxyribozymes that catalyze the Diels−Alder reaction.

Separately using the DAR and DAB pools, we performed in vitro selection as illustrated in Figure 1.[18] The selection process was initiated by primer extension on a DNA template using Taq polymerase and a DNA oligonucleotide primer with anthracene attached at the 5′-end via a hexaethylene glycol (HEG) tether. Each selection round consisted of three iterated steps: (1) Incubation with DTME (dithiobismaleimidoethane) to allow the Diels−Alder reaction to proceed [the key selection step]; (2) Treatment with a 5′-thiol-DNA to attack the unreacted maleimide moiety of DTME, followed by PAGE separation of the extended DNA strands; and (3) PCR amplification to regenerate the anthracene-tethered DNA pool, now enriched in catalytically active sequences. In each round, the key selection step used incubation conditions of 100 μM DTME in 50 mM Tris, pH 7.5, 200 mM Na^+, and 100 mM K^+ with 20 mM each Mg^{2+} and Ca^{2+} along with 5 μM each Mn^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} (all as Cl^- salts) at 30 °C for 1 h; these were the same ion concentrations used during the original ribozyme selection.[8]

After 10 selection rounds, robust activities of 49% (DAR) and 33% (DAB) were observed.[18] After two additional rounds using only a 5-min incubation during the Diels−Alder selection step (leading to DAR and DAB activities of 18% and 13%), both round 12 pools were cloned, and individual deoxyribozyme clones were screened for catalytic activity. Many active sequences were found in both selection pools.[18] One particular clone, DAB22, also showed catalytic activity when tested *in trans* using the anthracene-HEG small-molecule substrate that was not covalently tethered to DNA (i.e., Anthr-HEG). DAB22 had 13 mutations relative to the parent 39M49 sequence, but its mfold-predicted secondary structure revealed no apparent relationship to the parent ribozyme.[18] Therefore, although DAB22 originated from the biased pool, it is a new catalytic sequence.[19] The enantioselectivity of DAB22 was not

10.1021/ja7111965 CCC: $40.75 © 2008 American Chemical Society

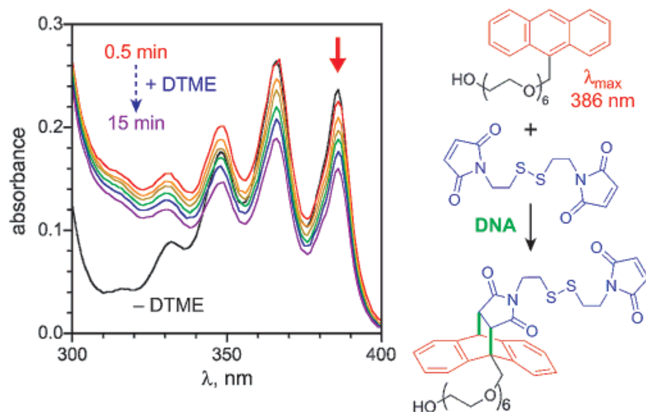

Figure 2. Diels–Alder catalysis by the DAB22 deoxyribozyme, as monitored by the decrease in anthracene absorbance upon reaction of Anthr-HEG with DTME. See text for kinetic values and Supporting Information for a complete description of the assays.

assayed experimentally; the enantiomer shown in Figure 1 is arbitrarily depicted as the same one formed by 39M49.

The *in trans* apparent second-order rate constant k_{app} of DAB22 was determined by monitoring the decreasing UV absorbance of anthracene, using Anthr-HEG and DTME as substrates (Figure 2).[20] Under standard conditions of 50 mM Tris, pH 7.5, and 23 °C, Ca^{2+} supports robust catalytic activity, as do Mg^{2+} and Mn^{2+}. In contrast, Co^{2+}, Cu^{2+}, and Zn^{2+} are ineffective, and Na^{+} and K^{+} are dispensable.[18] Under the standard conditions with 20 mM $CaCl_2$ and 10 μM deoxyribozyme, $k_{app} = 0.70 \pm 0.05$ M^{-1} s^{-1} ($K_{d,app}$ for Ca^{2+} of 5.5 ± 0.6 mM). This compares well with $k_{app} = 0.81 \pm 0.02$ M^{-1} s^{-1} that we determined for the 39M49 ribozyme under analogous conditions using the cognate substrate *N*-pentylmaleimide (NPM) and 80 mM Mg^{2+} (our value of k_{app} is essentially the same as that reported by Jäschke[8]). As detailed in the Supporting Information, the Michaelis–Menten parameters V_{max} and K_m are difficult to obtain separately for these nucleic acid enzymes as anything other than lower limits. Similar to observations for the 39M49 ribozyme, the K_m values for DTME and Anthr-HEG of >300 μM and >200 μM are rather large.

We determined the rate enhancement (RE) for the DAB22 deoxyribozyme in two ways: with the anthracene moiety either part of the free substrate molecule Anthr-HEG (*in trans*) or attached covalently to the DNA (*in cis*).[18] As described in the Supporting Information, the *in trans* product identity was confirmed by electrospray ionization mass spectrometry of the small-molecule reaction product after extraction with $CHCl_3$ from the aqueous sample. With anthracene *in trans*, the uncatalyzed rate constant k_{uncat} with DNA but without Ca^{2+} was relatively high, 0.056 M^{-1} s^{-1}, leading to a calculated RE = k_{app}/k_{uncat} of only 13 (similar values were obtained using k_{uncat} determined without DNA but with Ca^{2+}). Similarly, we determined the *in trans* RE for the 39M49 ribozyme as 25 with NPM and 15 with DTME, which are also very modest. In contrast, with anthracene attached to the DNA (*in cis*), we found a considerably higher RE = k_{obs}/k_{bkgd} of $>4 \times 10^5$ (k_{obs} 3 min^{-1} and $k_{bkgd} < 7 \times 10^{-6}$ min^{-1} at 50 μM DTME),[21] which is comparable to the observation *in cis* for 39M49 under analogous conditions (RE $> 1.5 \times 10^5$ and k_{obs} 5 min^{-1}).[18] Based on these rate constant and rate enhancement values, we conclude that DNA enzymes can be as efficient as the best known RNA enzymes for catalysis of the Diels–Alder reaction.

In summary, using in vitro selection we have identified new deoxyribozymes that catalyze the Diels–Alder reaction between anthracene and maleimide substrates. One of these deoxyribozymes, DAB22, was obtained from a biased pool that itself was derived from the previously reported 39M49 ribozyme, although the lack of any secondary structure relationship between DAB22 and 39M49 obviates sequence-function correlation.[22] As evidenced by comparison of both rate constants and rate enhancements, DAB22 catalyzes the Diels–Alder reaction as efficiently as 39M49, demonstrating experimentally that deoxyribozymes can be as efficient as the known ribozymes for catalysis of C–C bond formation. These data with small-molecule substrates show that the absence of 2′-hydroxyl groups in DNA relative to RNA is not an inherent impediment to robust catalytic function, consistent with findings that nucleic acids often rely on nucleobase functional groups as key catalytic components.[23] Our ongoing efforts seek to understand the mechanisms of deoxyribozyme catalysis and to expand the utility of DNA for catalyzing a variety of chemical reactions.

Acknowledgment. This research was supported by the National Institutes of Health (GM-65966 to S.K.S.). S.K.S. is a Fellow of the David and Lucile Packard Foundation.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kruger, K.; Grabowski, P. J.; Zaug, A. J.; Sands, J.; Gottschling, D. E.; Cech, T. R. *Cell* **1982**, *31*, 147–157. (b) Guerrier-Takada, C.; Gardiner, K.; Marsh, T.; Pace, N.; Altman, S. *Cell* **1983**, *35*, 849–857.
(2) Doudna, J. A.; Cech, T. R. *Nature* **2002**, *418*, 222–228.
(3) Joyce, G. F. *Annu. Rev. Biochem.* **2004**, *73*, 791–836.
(4) (a) Pan, T.; Uhlenbeck, O. C. *Biochemistry* **1992**, *31*, 3887–3895. (b) Bartel, D. P.; Szostak, J. W. *Science* **1993**, *261*, 1411–1418.
(5) (a) Johnston, W. K.; Unrau, P. J.; Lawrence, M. S.; Glasner, M. E.; Bartel, D. P. *Science* **2001**, *292*, 1319–1325. (b) Zaher, H. S.; Unrau, P. J. *RNA* **2007**, *13*, 1017–1026.
(6) (a) Tsukiji, S.; Pattnaik, S. B.; Suga, H. *J. Am. Chem. Soc.* **2004**, *126*, 5044–5045. (b) Tsukiji, S.; Pattnaik, S. B.; Suga, H. *Nat. Struct. Biol.* **2003**, *10*, 713–717.
(7) Tarasow, T. M.; Tarasow, S. L.; Eaton, B. E. *Nature* **1997**, *389*, 54–57.
(8) Seelig, B.; Jäschke, A. *Chem. Biol.* **1999**, *6*, 167–176.
(9) Silverman, S. K. *Wiley Encyclopedia of Chemical Biology* **2007**, in press.
(10) (a) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223–229. (b) Robertson, D. L.; Joyce, G. F. *Nature* **1990**, *344*, 467–468.
(11) (a) Peracchi, A. *ChemBioChem* **2005**, *6*, 1316–1322. (b) Höbartner, C.; Silverman, S. K. *Biopolymers* **2007**, *87*, 279–292.
(12) Cech, T. R. *Science* **1987**, *236*, 1532–1539.
(13) Silverman, S. K. *Nucleic Acids Res.* **2005**, *33*, 6151–6163.
(14) (a) Emilsson, G. M.; Nakamura, S.; Roth, A.; Breaker, R. R. *RNA* **2003**, *9*, 907–918. (b) Breaker, R. R.; Emilsson, G. M.; Lazarev, D.; Nakamura, S.; Puskarz, I. J.; Roth, A.; Sudarsan, N. *RNA* **2003**, *9*, 949–957.
(15) It is inherently impossible to assay the catalytic abilities of all possible deoxyribozymes and ribozymes, given the enormous size of sequence space for typical oligonucleotide lengths. The best that can be done is to compare the specific DNA and RNA enzymes that can in practice be identified.
(16) Serganov, A.; Keiper, S.; Malinina, L.; Tereshko, V.; Skripkin, E.; Höbartner, C.; Polonskaia, A.; Phan, A. T.; Wombacher, R.; Micura, R.; Dauter, Z.; Jäschke, A.; Patel, D. J. *Nat. Struct. Mol. Biol.* **2005**, *12*, 218–224.
(17) Stuhlmann, F.; Jäschke, A. *J. Am. Chem. Soc.* **2002**, *124*, 3238–3244.
(18) See Supporting Information for details of procedures and data analysis.
(19) Note that many DNA sequences substantially unrelated to the original RNA sequence were present in the biased pool at the outset of selection. For ~1% of the biased pool molecules, at least half of the nucleotides (18 or more out of 36) were different from those of the 39M49 ribozyme; for ~26% of the biased pool molecules, 13 or more differences were present. Therefore, we were not surprised to identify DNA sequences unrelated to the original ribozyme from the biased selection. See: Flynn-Charlebois, A.; Prior, T. K.; Hoadley, K. A.; Silverman, S. K. *J. Am. Chem. Soc.* **2003**, *125*, 5346–5350.
(20) The *in trans* reaction is technically termolecular, DNA + Anthr-HEG + DTME, with both of the latter compounds at subsaturating concentrations. The k_{app} is calculated as an apparent second-order rate constant at a fixed, known DNA concentration.
(21) The *in cis* reaction is technically bimolecular. The k_{obs} is the observed first-order rate constant at a fixed, subsaturating DTME concentration.
(22) Paul, N.; Springsteen, G.; Joyce, G. F. *Chem. Biol.* **2006**, *13*, 329–338.
(23) Bevilacqua, P. C.; Yajima, R. *Curr. Opin. Chem. Biol.* **2006**, *10*, 455–464.

JA7111965