DNA and RNA Can Be Equally Efficient Catalysts for Carbon–Carbon Bond Formation
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Catalysis by nucleic acids was merely a theoretical possibility until the discovery of catalytic RNAs (ribozymes) in the early 1980s. Although a variety of natural ribozymes have since been identified, 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. The repertoire of artificial ribozymes discovered in this fashion encompasses many chemical reactions including phosphodiester cleavage and ligation, RNA polymerization, redox reactions, carbon–carbon bond formation (Diels–Alder reaction), and many others. Because DNA catalysts were identified later than RNA 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. An early speculation was that the lack of 2′-hydroxyl groups in DNA would likely impair its catalytic efficiency relative to RNA, 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, suggest that RNA and DNA should be equally competent, although in both cases the highest theoretically possible rate enhancements have likely not been achieved. 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 began by considering the Diels–Alder ribozyme that was identified by Jäschke and co-workers using in vitro selection. 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, and the scope of substrate tolerance has been explored. One minimal form of this Diels–Alder ribozyme, 39M49, has 49 nucleotides. 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 (N40) 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.

Separately using the DAR and DAB pools, we performed in vitro selection as illustrated in Figure 1. 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 (dithiobismaleimidothioate) 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 Mg2+ and Ca2+ along with 5 μM each Mn2+, Co2+, Cu2+, and Zn2+ (all as Cl− salts) at 30 °C for 1 h; these were the same ion concentrations used during the original ribozyme selection.

After 10 selection rounds, robust activities of 49% (DAR) and 33% (DAB) were observed. 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. 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. Therefore, although DAB22 originated from the biased pool, it is a new catalytic sequence. The enantioselectivity of DAB22 was not...
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 kapp of DAB22 was determined by monitoring the decreasing UV absorbance of anthracene, using Anthr-HEG and DTME as substrates (Figure 2).

Under standard conditions of 50 mM Tris, pH 7.5, and 23 °C, Ca2+ supports robust catalytic activity, as do Mg2+ and Mn2+. In contrast, Co2+, Cu2+, and Zn2+ are ineffective, and Na+ and K+ are dispensable. Under the standard conditions with 20 mM CaCl2 and 10 μM deoxyribozyme, kapp = 0.70 ± 0.05 M−1 s−1 (Kapp for Co2+ of 5.5 ± 0.6 mM). This compares well with kapp = 0.81 ± 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 Mg2+ (our value of kapp is essentially the same as that reported by Jäschke12). As detailed in the Supporting Information, the Michaelis–Menten parameters Vmax and km are difficult to obtain separately for these nucleic acid enzymes as anything other than lower limits. Similar to observations for the 39M49 ribozyme, the km 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 covalently to the DNA (ki) or as the product of the catalysis (kobs).

Under the conditions of Figure 2, the catalysis of the Diels–Alder reaction between 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.

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

References

(23) Supporting Information: Details of procedures and data analysis.
(24) Note that many DNA sequences substantially unrelated to the original RNA sequence were present in the yield of the reaction. For ~1% of the cases, 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 yield of the following molecules, 13 or more differences were present. Therefore, we were not surprised to identify RNA sequences unrelated to the original ribozyme from the observed reaction. See: Flynn-Charlebois, A.; Prior, T. K.; Hoadley, K. A.; Silverman, S. K. J. Am. Chem. Soc. 2003, 125, 5346–5350.
(25) The in trans reaction is technically thermodynamic, DNA + Anthr-HEG + DTME, with both of the latter compounds at saturating concentrations.
(26) See text for kinetic values and Supporting Information, the Michaelis–Menten parameters Vmax and km are difficult to obtain separately for these nucleic acid enzymes as anything other than lower limits. Similar to observations for the 39M49 ribozyme, the km values for DTME and Anthr-HEG of 300 μM and >200 μM are rather large.
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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.